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TRANSMITTAL FORM

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Total Number of Pages in This Submission

Application Number	09/739,933
Filing Date	December 18, 2000
First Named Inventor	REID, JAMES STEVEN
Group Art Unit	1647
Examiner Name	TURNER, SHARON L.
Attorney Docket Number	UCAL-263CIP

ENCLOSURES (check all that apply)

- [X] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment / Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Documents
- [ ] Response to Missing Parts/ Incomplete Application
  - [ ] Response to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Assignment Papers *(for an Application)*
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s
- [ ] After Allowance Communication to Group
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to Group *(Appeal Notice, Brief, Reply Brief)*
- [ ] Proprietary Information
- [ ] Status Letter
- [X] Other Enclosure(s) *(please identify below):*
  1) **Appellants' Brief (48 pgs. – in triplicate)**
  2) **Exhibit 1**
  3) **Exhibit 2**
  4) **Return Postcard**

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Signing Attorney/Agent (Reg. No.)	PAULA A. BORDEN, 42,344 BOZICEVIC, FIELD & FRANCIS LLP
Signature	
Date	March 9, 2004

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FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 165.00

Complete if Known	
Application Number	09/739,933
Filing Date	December 18, 2000
First Named Inventor	REID, JAMES STEVEN
Examiner Name	TURNER, SHARON L.
Art Unit	1647
Attorney Docket No.	UCAL-263CIP

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ Other ☐ None

☒ Deposit Account:

Deposit Account Number: 50-0815

Deposit Account Name: Bozicevic, Field & Francis LLP

The Director is authorized to: (*check all that apply*)

☒ Charge fee(s) indicated below ☒ Credit any overpayments

☒ Charge Any Additional Fee(s) Required under 37 C.F.R. 1.17.

☐ Charge fee(s) indicated below, **except for the filing fee to the above-identified deposit account.**

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	
				SUBTOTAL (1)	0.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below		Fee Paid
Total Claims	-20** =		x	=	
Indep. Claims	-3** =		x	=	
Multiple Dependent				=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) $ 0.00

***or number previously paid, if greater; For Reissues, see above.*

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge – late filing fee or oath	
1052	50	2052	25	Surcharge – late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examination action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	165.00
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unavoidable	
1453	1,330	2453	665	Petition to revive – unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1406	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR § 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid **SUBTOTAL (3) ($)** 165.00

SUBMITTED BY		Complete (*if applicable*)			
Name (Print/Type)	Paula A. Borden	Registration No. (Attorney/Agent)	42,344	Telephone	(650) 833-7710
Signature				Date	03/09/2004

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APPELLANTS' BRIEF	Attorney Docket Confirmation No.	UCAL263CIP 4882
	First Named Inventor	J.S. Reid
	Application Number	09/739,933
	Filing Date	December 18, 2000
	Group Art Unit	1647
Address to: Mail Stop Appeal Brief-Patents Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Examiner Name	S.L. Turner
	Title	*Compositions and methods for manipulating glial progenitor cells and treating neurological deficits*

Sir:

This Brief is filed in support of Appellants' appeal from the Examiner's Rejection dated December 2, 2003. No claims have been allowed, and claims 1-3, 5-8, 33, 63, and 64 are pending. Claims 1-3, 5-8, 33, 63, and 64 are appealed. A Notice of Appeal was filed on January 9, 2004.

The Board of Appeals and Interferences has jurisdiction over this appeal pursuant to 35 U.S.C. §134.

The Commissioner is hereby authorized to charge deposit account number 50-0815 in the amount of $165.00 to cover the fee required under 37 C.F.R. §1.17(c) for filing Appellants' brief. In the unlikely event that the fee transmittal or other papers are separated from this document and/or other fees or relief are required, appellants petition for such relief, including extensions of time, and authorize the Commissioner to charge any fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 which may be required by this paper, or to credit any overpayment, to Deposit Account No. 50-0815, order number UCAL263CIP.

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REAL PARTY IN INTEREST

The inventors named on this patent application. The inventors assigned their entire rights to the invention to The Regents of the University of California.

RELATED APPEALS AND INTERFERENCES

There are currently no other appeals or interferences known to Appellant, the undersigned Appellants' representative, or the assignee to whom the inventors assigned their rights in the instant case, which would directly affect or be directly affected by, or have a bearing on the Board's decision in the instant appeal.

STATUS OF THE CLAIMS

This application is a continuation-in-part of U.S. Patent Application No. 09/129,028, filed August 4, 1998, which application claims benefit of priority to U.S. Provisional Patent Application No. 60/055,383, filed August 4, 1997.

Claims 1-62 were originally filed on December 18, 2000. Following a Restriction Requirement dated March 27, 2002, and a response thereto filed April 30, 2002, claims 9-15, 17-19, 21-26, 28-32, and 34-62 were withdrawn from consideration. In the response, filed on November 26, 2002 to the August 5, 2002 Office Action, claim 27 was canceled without prejudice to renewal; claims 1, 20, and 33 were amended; and new claims 63 and 64 were added. In the response, filed on July 22, 2003 to the April 22, 2003 Final Office Action, claims 4, 9-19, 21-32, and 34-62 were canceled without prejudice to renewal; and claims 1, 2, 20, 33, and 63 were amended. In the present Appeal Brief, claims 2, 3, and 5 are amended; and claim 20 is canceled without prejudice to renewal.

As a result of the amendments discussed above, claims 1-3, 5-8, 33, 63, and 64 remain pending.

All of the pending claims 1-3, 5-8, 33, 63, and 64 shown in attached Appendix I remain pending, rejected, and appealed here.

STATUS OF AMENDMENTS

During the course of prosecution, the following amendments were made. In an amendment, filed on November 26, 2002 and responsive to the August 5, 2002 Office Action, claim 27 was canceled; claims 1, 20, and 33 were amended; and new claims 63 and 64 were added. The November 26, 2002 amendments were entered. In an amendment, filed on July 22, 2003 and responsive to the April 22, 2003 Final Office Action, claims 4, 9-19, 21-32, and 34-62 were canceled; and claims 1, 2, 20, 33, and 63 were amended. The July 22, 2003 amendments were not entered. In a Request for Continued Examination filed on September 15, 2003, Applicants requested entry of the amendments presented in the July 22, 2003 amendment. The July 22, 2003 amendments were subsequently entered.

Current claim amendments

Claims 2, 3, and 5 are amended to address the rejection of these claims under 35 U.S.C.§112, second paragraph. Claim 20 is canceled without prejudice to renewal, and is not appealed here.

As discussed in the Manual of Patent Examining Procedure §1207, an amendment filed at any time after final rejection, but before jurisdiction has passed to the Board may be entered upon or after filing of an appeal brief provided that the amendment conforms to the requirements of 37 C.F.R.§1.116. Appellants submit that the amendments to claims 2, 3, and 5 conform to the requirements of 37 C.F.R.§1.116. As stated in 37 C.F.R.§1.116, an amendment after final or appeal may be made canceling claims, complying with any requirement of form expressly set forth in a previous Office Action, or placing rejected claims in better form for allowance. The amendments to claims 2, 3, and 5 are made to address a rejection under 35 U.S.C.§112, second paragraph, which noted that the claim language of claims 2, 3, and 5 does not track the claim language of claim 1, from which claims 2, 3, and 5 depend. The amendments to claims 2, 3, and 5 thus place these rejected claims in better form for allowance. As such, the amendments to claims 2, 3 and 5, and the cancellation of claim 20 without prejudice are admissible and should be entered.

SUMMARY OF THE INVENTION

The instant invention provides methods for attracting a neural progenitor cell or a progeny thereof to a site of damage or lesion in the central nervous system (CNS). The methods as claimed involve administration of a transforming growth factor-alpha (TGF-α) polypeptide or a functional fragment thereof to a site outside the ventricles in an individual having a CNS lesion or damage. The methods are useful for treating various neurological disorders, including neurodegenerative disorders, such as Parkinson's Disease. Specification, page 11, lines 26-29.

The inventors made several observations, which are illustrated graphically in the accompanying Figure (provided herewith as Exhibit 1), which is a modification of Figure 1 as filed with the application.[1] In short, the inventors found that:

- Administration of a TGF-α polypeptide outside the ventricles in the absence of CNS damage or lesion does not induce migration of neural progenitor cells or progeny from the ventricles. Specification, page 71, lines 23-25; and **Exhibit 1, Panel A**.
- Administration of a TGF-α polypeptide into the ventricles, even in the presence of a CNS lesion, does not does not induce migration of neural progenitor cells or progeny from the ventricles. Specification, page 57, lines 8-11; page 72, lines 17-19; and **Exhibit 1, Panel B**.
- The presence of a CNS lesion, without administration of a TGF-α polypeptide, does not does not induce migration of neural progenitor cells or progeny from the ventricles. Specification, page 54, lines 12-14; and page 57, lines 22-29; page 71, lines 20-23; and **Exhibit 1, Panel C**
- Administration of a TGF-α polypeptide outside the ventricles, in the presence of a CNS lesion, induces mass migration of neural progenitor cells and progeny thereof directed toward the site of the CNS lesion. Specification, page 12, lines 3-10; page 54, lines 18-20; page 55, lines 15-26; page 75, lines 9-18; and Figure 9; **Exhibit 1, Panel D**.

[1] The features shown in the accompanying Figure are not necessarily to scale; rather, they are intended to provide a schematic illustration of the results observed by the inventors.

The invention is based in part on experimental observations made by the inventors.[2] The following is a summary of the experimental observations. An amount of 6-hydroxydopamine (6-OHDA) was injected into the substantia nigra of brains of adult rats, which induced brain lesions.[3] TGF-α was then administered to the midstriatal region of the brains of the animals, a location which is outside the ventricles.[4]

The results showed that:

- Infusion of TGF-α intrastrially (i.e., outside the ventricles), combined with the presence of a 6-OHDA-induced brain lesion, induced formation of a dense ridge of cells in the striatum ("the striatal ridge"), which cells abundantly expressed EGF receptor mRNA, and **migrated** away from the ventricles and toward the lesion. [5] Specification, page 48, lines 25-30; page 49, lines 15-19; page 50, lines 29-31; page 55, lines 10-11; page 56, lines 9-10; page 68, lines 4-10.
- Infusion of TGF-α, in the absence of brain lesion, did not result in formation of the striatal ridge, and thus did not induce migration of neural progenitor cells away from the ventricles. Specification, page 46, lines 20-21; page 71, lines 23-25. Similarly, in control animals that were given CNS lesions but no TGF-α, there was no migration of neural progenitor cells away from the ventricles. Specification, page 71, lines 20-23.
- Striatal infusions (i.e., administration outside the ventricles) of TGF-α, when combined with nigral 6-OHDA lesions, induce the formation of the striatal ridge in the body of the striatum. Specification, page 48, lines 25-30; and page 49, lines 15-16. When the cells forming this striatal ridge were characterized, it was found that the striatal ridge includes **neural progenitors**. Specification, page 61, lines 7-20; and page 62, Table 2.
- When TGF-α was administered into the ventricles, the striatal ridge did not form, indicating that there was no migration of neural progenitor cells away from the ventricles. Specification, page 57, lines 8-11; and page 72, lines 17-19.

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[2] Specification, page 11, line 30 to page 12, line 10.
[3] Specification, page 41, lines 1-12.
[4] Specification, page 41, lines 14-23.
[5] TGF-α binds to the EGF receptor (EGF-R). TGF-α-responsive neural progenitor cells express EGF-receptor mRNA; thus

- In animals with both induced brain lesion and infusion of TGF-α outside the ventricles, there was a rapid proliferation of forebrain stem cells followed by a timed migration of a ridge of neuronal and glial progenitor cells directed toward the region of the TGF-α infusion site. Over time, increasing numbers of differentiated neurons were observed in the striatum. Specification, page 55, line 15 to page 56, line 2; and page 59, lines 13-17.
- The migration of neural progenitor cells, induced by the combination of a brain lesion and administration of TGF-α, correlated with **improved brain function**. Specification, page 73, line 16 to page 75, line 18. The inventors used a standard test, i.e., rotational behavior in response to systemically administered apomorphine, to determine the effect of TGF-α on function. Specification, page 73, lines 21-25. In behavioral experiments, there was a significant reduction of apomorphine-induced rotations in animals receiving the TGF-α infusions. Specification, page 74, lines 8-10.

Thus, the combination of the injury signal (CNS damage or lesion) and administration of a TGF-α polypeptide to a site outside the ventricles resulted in *therapeutically significant* proliferation and directed migration of progenitor cells (or their progeny) away from the ventricles and toward the site of injury.

EGF-R mRNA expression was sometimes used to detect the striatal ridge.

ISSUES

There are three issues on appeal, as follows:

I. WHETHER CLAIMS 2, 3, 5, AND 20 COMPLY WITH THE REQUIREMENTS OF 35 U.S.C. §112, SECOND PARAGRAPH;

II. WHETHER THE INVENTION IS ANTICIPATED UNDER 35 U.S.C. §102(e) BY U.S. PATENT NO. 5,980,885; AND

III. WHETHER THE INVENTION IS OBVIOUS UNDER 35 U.S.C. §103(a) IN VIEW OF U.S. PATENT NO. 5,980,885.

GROUPING OF THE CLAIMS

Claims 1-3, 5-8, 33, 64, and 65 are directed to methods for attracting a neural progenitor cell and are argued as a group. With respect to the rejection under 35 U.S.C.§102(e) and the rejection under 35 U.S.C.§103(a) as set forth in the December 2, 2003 Office Action, claims 1-3, 5-8, 33, 64, and 65 are argued as a group and stand or fall together.

ARGUMENTS

The arguments portion of this Brief is divided into two sections. The first section describes Appellants' understanding of the Examiner's rejections. The second section specifically addresses the three issues outlined above relating to compliance of the claimed invention with the requirements of 35 U.S.C. §112, second paragraph; the novelty of the invention over U.S. Patent No. 5,980,885; and the non-obviousness of the invention in view of .S. Patent No. 5,980,885.

THE EXAMINER'S REJECTIONS

Rejection under 35 U.S.C.§112, second paragraph

Claims 2, 3, 5, and 20 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. The December 2, 2003 Office Action stated that that claim 1 recites particular administration *in vivo* to an individual; and further stated that claim 20 recites that the CNS tissue is in tissue culture and thus the administration is apparently not *in vivo*. The Office Action further stated that claims 2, 3, 5, and 20 recite the

limitation "the compound" in reference to claim 1; and further stated that there is insufficient basis for this phrase. Claim 20 is canceled without prejudice to renewal, thereby rendering the rejection of this claim moot. Appellants submit that the amendments to claims 3 and 5, as shown in the Appendix, adequately address this rejection.

Rejection under 35 U.S.C.§102(e)

Claims 1-3, 5-8, 20, 33, 63, and 64 were rejected under 35 U.S.C. §102(e) as allegedly anticipated by U.S. Patent No. 5,980,885 ("Weiss"). In support of this rejection, the Office argued that Weiss teaches administration of TGF-α to patients *in vivo* for the purpose of inducing *in vivo* proliferation, migration, and differentiation of neural and/or glial cell precursors and for treatment of injuries and diseases of the nervous system including Huntington's, Alzheimer's, Parkinson's, and other neurological disorders. The Office also argued that Weiss is not limited to ventricular administration of growth factor.

Rejection under 35 U.S.C.§103

Claims 1-3, 5-8, 20, 33, 63, and 64 were rejected under 35 U.S.C. §103 as allegedly obvious in view of Weiss. In support of this rejection, the Office argued that, while Weiss fails to *ipsis verbis* teach administration of TGF-α outside the ventricles, via intrastriatal administration, and wherein the site is spinal cord tissue and spinal nerve root origins, such limitations are rendered obvious by the reference as a whole.

APPELLANTS' RESPONSE TO THE REJECTIONS

Rejection under 35 U.S.C.§112, second paragraph

The rejection of claims 2, 3, 5, and 20 under 35 U.S.C. §112, second paragraph, is adequately addressed by the amendments to claims 2, 3, and 5. Claim 20 is canceled without prejudice to renewal, thereby rendering the rejection of this claim moot.

Rejection under 35 U.S.C.§102(e)

The rejection of claims 1-3, 5-8, 20, 33, 63, and 64 under 35 U.S.C.§102(e) is in error. Weiss does not disclose a method as claimed, comprising administering a TGF-α polypeptide or a functional fragment thereof to an individual having CNS damage or lesion, where the TGF-α polypeptide or functional fragment thereof is administered outside the ventricles. Weiss does not disclose administration of a TGF-α polypeptide or a functional fragment thereof to a site outside the ventricles in an individual having a CNS lesion or damage. Weiss does not teach a method involving administration of a TGF-α polypeptide or functional fragment thereof, such that migration of a neural progenitor cell or progeny to a site of CNS lesion or damage is effected. Weiss does not teach administering a TGF-α polypeptide or functional fragment thereof to an individual having a CNS damage or lesion. Weiss does not teach each and every claim element. Accordingly, Weiss cannot anticipate the appealed claims.

Rejection under 35 U.S.C.§103

The rejection of claims 1-3, 5-8, 20, 33, 63, and 64 under 35 U.S.C.§103 is in error. Weiss does not provide motivation to modify the teachings of Weiss to administer a TGF-α polypeptide to a site outside the ventricles; Weiss does not provide a reasonable expectation of success; and Weiss does not teach or suggest all of the claim limitations. Accordingly, Weiss cannot render the appealed claims obvious.

I. WHETHER CLAIMS 2, 3, 5, AND 20 COMPLY WITH THE REQUIREMENTS OF 35 U.S.C. §112, SECOND PARAGRAPH

Claim 20

The December 2, 2003 Office Action stated that claim 1 recites particular administration *in vivo* to an individual, and that claim 20 specifies that the CNS tissue is in tissue culture and thus the administration is not apparently *in vivo*. Without conceding as to the correctness of this rejection, claim 20 is canceled without prejudice to renewal.

Claims 2, 3, and 5

The December 2, 2003 Office Action stated that claims 2, 3, and 5 recite the limitation "the compound" in reference to claim 1; and further stated that there is insufficient antecedent basis for "the compound."

Claims 2, 3, and 5 are amended to delete "the compound," and to substitute therefor "the TGF-α polypeptide or functional fragment thereof." The phrase "the TGF-α polypeptide or functional fragment thereof" is recited in claim 1, from which claims 2, 3, and 5 depend. Thus, claims 2, 3, and 5 as amended track the language recited in claim 1. Accordingly, claims 2, 3, and 5 as amended comply with the requirements of 35 U.S.C.§112, second paragraph.

The amendments to claims 2, 3, and 5 place the claims in better form for allowance. As discussed in the Manual of Patent Examining Procedure §1207, an amendment filed at any time after final rejection, but before jurisdiction has passed to the Board may be entered upon or after filing of an appeal brief provided that the amendment conforms to the requirements of 37 C.F.R.§1.116. Appellants submit that the amendments to claims 2, 3, and 5 conform to the requirements of 37 C.F.R.§1.116. As stated in 37 C.F.R.§1.116, an amendment after final or appeal may be made canceling claims, complying with any requirement of form expressly set forth in a previous Office Action, or placing rejected claims in better form for allowance. The amendments to claims 2, 3, and 5 are made to address a rejection under 35 U.S.C.§112, second paragraph, which noted that the claim language of claims 2, 3, and 5 does not track the claim language of claim 1, from which claims 2, 3, and 5 depend. The amendments to claims 2, 3, and 5 thus place these rejected claims in better form for allowance. As such, the amendments to claims 2, 3, and 5, and the cancellation of claim 20 without prejudice are admissible and should be entered.

II. WHETHER THE INVENTION IS ANTICIPATED UNDER 35 U.S.C. §102(e) BY U.S. PATENT NO. 5,980,885

The December 2, 2003 Office Action rejected claims 1-3, 5-8, 20, 33, 63, and 64 under 35 U.S.C.§102(e) as allegedly anticipated by U.S. Patent No. 5,980,885 ("Weiss").

The December 2, 2003 Office Action stated:

(1) Weiss teaches administration of TGF-α to patients *in vivo* for the purpose of inducing *in vivo* proliferation, migration and differentiation of neural and/or glial cell precursors and for the treatment of injuries and diseases of the nervous system including Huntington's, Alzheimer's, Parkinson's and other neurological disorders;

(2) Weiss is not limited to ventricular administration of growth factor; and

(3) Weiss establishes that the treatment is to an individual having CNS damage or lesion. The December 2, 2003 Office Action stated that the Weiss patent fails to *ipsis verbis* teach administration of TGF-α outside the ventricles, via intrastriatal administration, and wherein the site is spinal cord tissue and spinal nerve root origins. The December 2, 2003 Office Action stated that such limitations are anticipated by Weiss.

The claims on appeal are directed to:

1) parenteral administration of a TGF-α polypeptide or a functional fragment thereof to an individual having CNS damage or lesion, where the TGF-α administered outside the ventricles so as to effect migration of neural progenitor cells (or progeny thereof) to the site of CNS damage or lesion (independent claim 1);

2) administration of a TGF-α polypeptide or a functional fragment thereof to a site of CNS damage or lesion in an individual, where the TGF-α is administered outside the ventricles, so as to attract neural progenitor cells (or progeny thereof) to the site of CNS damage or lesion (independent claim 33); and

3) administration of a TGF-α polypeptide or a functional fragment thereof to a site of CNS damage or lesion in an individual, where the TGF-α administered intrastriatially (*i.e.*, to a site outside the ventricles), so as to attract neural progenitor cells (or progeny thereof) to the site of CNS damage or lesion (independent claim 63).

In contrast, Weiss discusses methods of increasing the numbers of neural stem cell progeny, so that the neural stem cell progeny can be harvested and used for neurotransplantation into a host. Weiss, column 11, lines 42-66. As part of the method, Weiss discusses introducing a growth factor to the ventricles or into the ventricles of a mammalian host, to increase the numbers of neural stem cell progeny in the ventricles. It should be noted that Weiss defines "ventricle" as "any cavity or passageway within the CNS through which cerebral spinal fluid flows" (which would include the lumbar cistern); furthermore, Weiss states that the term "ventricle" encompasses the lateral, third, and fourth ventricles, and the central canal, cerebral aqueduct, and other CNS cavities. Weiss, column 13, lines 4-11.

The "Detailed Description" section of Weiss is divided into the following sections:

1) "Multipotent Neural Stem Cells"
This section discusses the properties of multipotent neural stem cells. Weiss, column 11, line 67 to column 15, line 4;

2) "In Vitro Proliferation of Neural Stem Cells"
This section discusses *in vitro* culture conditions for generating neural stem cell progeny from cells taken from the brain, and *in vitro* modification of the cells. Weiss, column 15, line 5 to column 21, line 48;

3) "Transplantation of Neural Stem Cell Progeny Alleviate Disorders of the CNS in Animal Models Caused by Disease or Injury"
This section discusses transplantation of neural stem cell progeny into the brain. Weiss, column 21, line 49 to column 25, line 20.

4) "In Vivo Proliferation, Differentiation, and Genetic Modification of Neural Stem Cell Progeny"

This section discusses techniques to induce *in vivo* proliferation, differentiation, and genetic modification of neural stem cell progeny; and indicates that growth factors should be administered to the ventricles. Weiss, column 25, line 21 to column 28, line 26;

5) "In Vitro Models of CNS Development, Function and Dysfunction, and Methods for Screening Effects of Drugs on Neural Cells"
This section discusses use of neural stem cell progeny in screening methods to test the effect of an agent on neural stem cell progeny. Weiss, column 28, line 26 to column 32, line 39; and

6) Examples 1-45.

As discussed in more detail below, nowhere in any of the above-noted sections does Weiss disclose or suggest a method for attracting a neural progenitor cell, or a progeny thereof, to a site of damage or lesion in a CNS tissue, the method comprising administering to an individual having CNS damage or lesion, wherein the administration is outside the ventricles, a TGF-α polypeptide or a functional fragment thereof, such that the administration effects migration of the neural progenitor cell or progeny thereof to the site of damage or lesion in the CNS tissue.

As noted above, the instant invention as claimed reflects the observations that:

a) Both the presence of a CNS damage or lesion and administration of a TGF-α polypeptide or a functional fragment thereof are required for **migration** of neural progenitor cells or their progeny away from the ventricles and toward a site of CNS damage or lesion; and

b) The TGF-α polypeptide (or functional fragment thereof) must be administered to a site outside the ventricles. Administration of TGF-α to the ventricles does not result in substantial migration of neural progenitor cells or their progeny away from the ventricles.

Administration of a growth factor to or into the ventricles as taught by Weiss simply does not provide this combination. The combination is neither taught nor suggested by Weiss. Instead, Weiss teaches, and particularly in the context of treatment that involves CNS damage or lesion, growth factors are to be administered into the ventricles (see, Weiss at e.g., col. 26, lines 15-26).

During an in-person interview,[6] Dr. Fallon explained that administration of a growth factor such as TGF-α into the ventricles as taught by Weiss is not effective to stimulate migration of a neural progenitor cell or progeny thereof to a site of damage or lesion in the CNS. Instead, the TGF-α must be administered to a site outside the ventricles. Dr. Fallon further explained that administration of a growth factor such as TGF-α alone, in the absence of CNS damage or lesion, is ineffective to stimulate migration of a neural progenitor cell or progeny thereof. Instead, both administration of a growth factor such as TGF-α (to a site outside the ventricles) and the presence of CNS damage or lesion are required to stimulate migration of a neural progenitor cell or progeny thereof to a site of damage or lesion in the CNS.

As Dr. Fallon noted during the Examiner Interview, the fact that CNS damage or lesion must be present in order to stimulate migration of a neural progenitor cell or progeny is discussed in Fallon et al. ((2000) *Proc. Natl. Acad. Sci. USA* 97:14686-14691; a copy of which was provided in the response filed on July 22, 2003, and is provided herewith as Exhibit 2 as a courtesy.)

During the interview, Dr. Fallon also explained that TGF-α administration into the ventricles simply does not induce production of a therapeutically significant proliferation of progenitor cells, and does not provide for detectable migration toward the site of injury. As Dr. Fallon explained, there are a number of barriers between the ventricles and the brain tissue outside the ventricles. Neural progenitor cells resident within the ventricles normally migrate through the ventricles toward the olfactory bulb, and do not typically migrate through these barriers into the brain tissue outside the ventricles.

[6] An in-person interview took place on June 25, 2003, and was attended by Examiners Turner and Kunz, inventor Dr. James Fallon, and Applicants' representatives Carol L. Francis and Paula A. Borden.

Weiss does not anticipate the instant invention as claimed

It is basic patent law that in order to anticipate a claim, a single prior art reference must disclose every essential element of the invention.[7] Anticipation is a question of fact, and disclosure of each claimed element can be disclosed either explicitly or inherently.[8] Exclusion of one claimed element from a prior art reference is enough to negate anticipation by that reference.[9] Thus, a reference may only be anticipatory under 35 U.S.C. §102 if it discloses each claim element or a functional equivalent thereof.

Weiss fails to teach a method for attracting a neural progenitor cell or progeny thereof to a site of damage or lesion in a CNS tissue. Weiss fails to teach a method that requires both the presence of a CNS damage or lesion and administration of a TGF-α polypeptide or a functional fragment thereof. Weiss fails to teach a method that results in migration of neural progenitor cells or their progeny away from the ventricles and toward a site of CNS damage or lesion. Weiss fails to teach a method of inducing migration of neural progenitor cells, which method involves administration of a TGF-α polypeptide (or functional fragment thereof) to a site outside the ventricles.

Weiss fails to disclose each and every element of the instant invention as claimed. Accordingly, Weiss cannot anticipate the instant invention as claimed.

The December 2, 2003 Office Action

The December 2, 2003 Office Action acknowledged that Weiss fails to *ipsis verbis* teach administration of TGF-α "outside the ventricles," via "intrastriatal administration," and wherein the site is "spinal cord tissue and spinal root origins." December 2, 2003 Office Action, page 4.

The December 2, 2003 Office Action stated that Weiss teaches administration of TGF-α to patients *in vivo* for the purpose of inducing *in vivo* proliferation, migration, and differentiation of neural and/or glial cell

[7] *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 231 U.S.P.Q. (BNA) 81, 90 (Fed. Cir. 1986).
[8] *In re Schreiber*, 128 F.3d 1473, 1477 (Fed. Cir. 1997) (citation omitted); *Tyler Refrigeration v. Kysor Industrial Corp.*, 777 F.2d 687, 698, 227 U.S.P.Q. (BNA) 845, 846-47 (Fed. Cir. 1985).
[9] *Atlas Powder Co. v. E.I. Du Pont de Nemours & Co.*, 750 F.2d 1569, 1574 224 U.S.P.Q. (BNA) 409, 411 (Fed.

precursors and for treatment of injuries and diseases of the nervous system, including Huntington's, Alzheimer's, Parkinson's and other neurological disorders. As support, the December 2, 2003 Office Action cited the Abstract, lines 5-7; column 25, line 20 to column 26, line 64; and Examples 27-30 of Weiss.

Each of these portions of the Weiss patent is reviewed in turn.

The Abstract

[57] **ABSTRACT**

A method is described for inducing in vivo proliferation of precursor cells located in mammalian neural tissue by administering to the mammal a fibroblast growth factor and at least one additional growth factor selected from the group consisting of epidermal growth factor, transforming growth factor alpha, and amphiregulin. The method can be used to replace damaged or missing neurons and/or glia. Another method is described for transplanting multipotent neural stem cell progeny into a mammal. The method comprises the steps of administering growth factors to a mammal to induce in vivo proliferation of neural precursor cells, removing the precursor cell progeny from the mammal, culturing the removed cells in vitro in the presence of one or more growth factors that induces multipotent neural stem cell proliferation, and implanting the multipotent neural stem cell progeny into the mammal.

The Weiss Abstract states that a method is described for inducing *in vivo* proliferation of precursor cells located in a mammalian neural tissue **by administering to the mammal a fibroblast growth factor and at least one additional growth factor**. (Note that there is no discussion of induction of migration.) The Abstract further states that another method is described for transplanting multipotent neural stem cell progeny into a mammal, the method comprising the steps of **administering growth factor to a mammal; removing the precursor cell progeny from the mammal, culturing the removed cells *in vitro* in the presence of one or more growth factors; and implanting the multipotent neural stem cells into the mammal.**

Cir. 1984).

In contrast, the instant invention as claimed comprises administering to a site outside the ventricles a TGF-α polypeptide or a functional fragment thereof. The Abstract does not disclose or suggest such a method.

Column 25, line 20 to column 26, line 64

In Vivo Proliferation, Differentiation, and Genetic Modification of Neural Stem Cell Progeny

Neural stem cells and their progeny can be induced to proliferate and differentiate in vivo by administering to the host, any growth factor(s) or pharmaceutical composition that will induce proliferation and differentiation of the cells. These growth factors include any growth factor known in the art, including the growth factors described above for in vitro proliferation and differentiation. Pharmaceutical compositions include any substance that blocks the inhibitory influence and/or stimulates neural stem cells and stem cell progeny to proliferate and ultimately differentiate. Thus, the techniques described above to proliferate, differentiate, and genetically modify neural stem cells in vitro can be adapted to in vivo techniques, to achieve similar results. Such in vivo manipulation and modification of these cells allows cells lost, due to injury or disease, to be endogenously replaced, thus obviating the need for transplanting foreign cells into a patient. Additionally, the cells can be modified or genetically engineered in vivo so that they express various biological agents useful in the treatment of neurological disorders.

Administration of growth factors can be done by any method, including injection cannula, transfection of cells with growth hormone-expressing vectors, injection, timed-release apparati which can administer substances at the desired site, and the like. Pharmaceutical compositions can be administered by any method, including injection cannula, injection, oral administration, timed-release apparati and the like. The neural stem cells can be induced to proliferate and differentiate in vivo by induction with particular growth factors or pharmaceutical compositions which will induce their proliferation and differentiation. Therefore, this latter method circumvents the problems associated with transplantation and immune reactions to foreign cells. Any growth factor can be used, particularly EGF, TGFα, FGF-1, FGF-2 and NGF.

Growth factors can be administered in any manner known in the art in which the factors may either pass through or by-pass the blood-brain barrier. Methods for allowing factors to pass through the blood-brain barrier include minimizing the size of the factor, or providing hydrophobic factors which may pass through more easily.

The fact that neural stem cells are located in the tissues lining ventricles of mature brains offers several advantages for the modification and manipulation of these cells in vivo and the ultimate treatment of various neurological diseases, disorders, and injury that affect different regions of the CNS. Therapy for these can be tailored accordingly so that stem cells surrounding ventricles near the affected region would

be manipulated or modified in vivo using the methods described herein. The ventricular system is found in nearly all brain regions and thus allows easier access to the affected areas. If one wants to modify the stem cells in vivo by exposing them to a composition comprising a growth factor or a viral vector, it is relatively easy to implant a device that administers the composition to the ventricle and thus, to the neural stem cells. For example, a cannula attached to an osmotic pump may be used to deliver the composition. Alternatively, the composition may be injected directly into the ventricles. The neural stem cell progeny can migrate into regions that have been damaged as a result of injury or disease. Furthermore, the close proximity of the ventricles to many brain regions would allow for the diffusion of a secreted neurological agent by the stem cells or their progeny.

(emphasis added)

For treatment of Huntington's Disease, Alzheimer's Disease, Parkinson's Disease, and other neurological disorders affecting primarily the forebrain, growth factors or other neurological agents would be delivered to the ventricles of the forebrain to affect in vivo modification or manipulation of the stem cells. For example, Parkinson's Disease is the result of low levels of dopamine in the brain, particularly the striatum. It would be advantageous to induce a patient's own quiescent stem cells to begin to divide in vivo and to induce the progeny of these cells to differentiate into dopaminergic cells in the affected region of the striatum, thus locally raising the levels of dopamine.

(emphasis added)

Normally the cell bodies of dopaminergic neurons are located in the substantia nigra and adjacent regions of the mesencephalon, with the axons projecting to the striatum. Prior art methods for treating Parkinson's disease usually involves the use of the drug L-Dopa, to raise dopamine levels in the striatum. However, there are disadvantages with this treatment including drug tolerance and side effects. Also, embryonic tissues that produce dopamine have been transplanted into the striatum of human Parkinsonian patients with reasonable success. However, the use of large quantities of fetal human tissue required for this procedure raises serious ethical concerns and practical issues.

The methods and compositions of the present invention provide an alternative to the use of drugs and the controversial use of large quantities of embryonic tissue for treatment of Parkinson's disease. Dopamine cells can be generated in the striatum by the administration of a composition comprising growth factors to the lateral ventricle. A particularly preferred composition comprises a combination of EGF, FGF-2, and heparan sulphate. The composition preferably also comprises serum. After administration of this composition, there is a significant increase in the transcription of messenger RNA (mRNA) for TH in the subventricular region of the striatum, an area which normally does not contain dopaminergic cell bodies. These methods and results are described in detail in Example 34. As detailed in Example 35, the use of dual labeling tissue to show the distribution of BrdU+ and TH+ cells indicates that, in response to the in vivo administration of growth factors, TH+ cell bodies occur in striatal tissue. Many of these newly generated TH+ cells are also BrdU+.

(emphasis added)

For the treatment of MS and other demyelinating or hypomyelinating disorders, and for the treatment of Amyotrophic Lateral Sclerosis or other motor neuron diseases, growth factors or other neurological agents would be delivered to the central canal.

In addition to treating CNS tissue immediately surrounding a ventricle, a viral vector, DNA, growth factor, or other neurological agent can be easily administered to the lumbar cistern for circulation throughout the CNS.

(emphasis added)

At column 25, line 20 to column 26, line 64, Weiss states: "If one wants to modify the stem cells *in vivo* by exposing them to a composition comprising a growth factor or a viral vector, it is relatively easy to implant a device that administers the compositions **to the ventricle** and thus, to the neural stem cells." Weiss, column 26, lines 4-8 (emphasis added). Weiss further states: "Alternatively, the composition may be injected directly **into the ventricles**." Weiss, column 26, lines 9-10 (emphasis added). Weiss further states: "Dopamine cells can be generated in the striatum by the administration of a composition comprising growth factors **to the lateral ventricle**." Weiss, column 26, lines 42-44. Weiss further states: "For the treatment of MS and other demyelinating or hypomyelinating disorders, and for the treatment of Amyotrophic Lateral Sclerosis or other motor neuron diseases, growth factors or other neurological agents would be delivered **to the central canal**."[10] Weiss, column 26, lines 57-60 (emphasis added). Thus, Weiss explicitly teaches to administer growth factor **to the ventricles**.

Thus, the entire emphasis of column 25, line 20 to column 26, line 64 of Weiss is on administration of growth factors **to the ventricles**.

Examples 27-30

EXAMPLE 27

In Vivo Proliferation of Neural Stem Cells of Lateral Ventricle

A replication incompetent retrovirus containing the β-galactosidase gene [as described in Walsh and Cepko,

[10] Weiss defines "ventricle" as "any cavity or passageway within the CNS through which cerebral spinal fluid flows" (which would include the lumbar cistern); furthermore, Weiss states that the term "ventricle" encompasses the lateral, third, and fourth ventricles, and the central canal, cerebral aqueduct, and other CNS cavities. Weiss, column 13, lines 4-11.

Science 241:1342, (1988)] was injected into the forebrain lateral ventricles of CD1 adult male mice (25–30 g from Charles River). The injected retrovirus was harvested from the BAG cell line (ATCC CRL-9560) according to the method of Walsh and Cepko (supra). Mice were anesthetized using 65 mg/kg, i.p. sodium pentobarbital. Unilateral stereotactic injections of 0.2–1.0 μl of retrovirus were injected into the lateral ventricle using a 1 μl Hamilton syringe. The coordinates for injection were AP +4.2 mm anterior to lambda, L±0.7 mm, and DV −2.3 mm below dura, with the mouth bar at −2 mm below the interaural line.

On the same day as, one day, or six days following the retrovirus injection, an infusion cannulae attached to a 0.5 μl/hour ALZET osmotic mini-pumps filled with 3.3–330 μg/ml of EGF were surgically implanted into the lateral ventricles at the identical stereotactic coordinates as stated above. The infusion cannula kits were obtained from ALZA. The infusion cannulae were cut to 2.7 mm below the pedestal. The pumps were secured to the mouse skull by use of acrylic cement and a skull screw contralateral and caudal to the injection site. The osmotic mini-pump was situated subcutaneously under and behind the armpit of the left front paw and connected to the infusion cannula by the means of polyethylene tubing.

(emphasis added)

Six days following initiation of EGF infusion the animals were sacrificed with an overdose of sodium pentobarbital. Mice were transcardially perfused with 2% buffered paraformaldehyde, and the brains were excised and post fixed overnight with 20% sucrose in 2% buffered paraformaldehyde. Coronal slices were prepared with −20 celsius cryostat sectioning at 30 μm. Slices were developed for β-gal histochemistry as per Morshead and Van der Kooy (supra).

Under these conditions, regardless of the day post retrovirus injection, infusion of EGF resulted in an expansion of the population of β-gal labelled cells from an average of 20 cells per brain up to an average of 150 cells per brain and the migration of these cells away from the lining of the lateral ventricles. Infusion of FGF-2 at 33 μg/ml resulted in an increase in the number of β-gal labelled cells, but this increase was not accompanied by any additional migration. Infusion of EGF and FGF together resulted in an even greater expansion of the population of β-gal labelled cells from 20 cells per brain to an average of 350 cells per brain.

These results indicate that FGF may be a survival factor for relatively quiescent stem cells in the subependyma layer, whereas EGF may act as a survival factor for the normally dying progeny of the constitutively proliferating population. The synergistic increase in β-galactosidase cell number when EGF and FGF are infused together further reflects the direct association between the relatively quiescent stem cell and the constitutively proliferating progenitor cell.

Example 27 discusses *in vivo* proliferation of neural stem cells of the lateral ventricle. A recombinant retrovirus containing a β-galactosidase gene ("β-gal retrovirus") was injected into the forebrain lateral ventricles of CD1 adult male mice. Weiss, column 46, line 66 to column 47, line 2. Osmotic minipumps filled with EGF were surgically implanted into the lateral ventricles on the same day that the β-gal retrovirus was injected. Weiss, column 47, lines 12-16. Six days following initiation of EGF infusion, the mice were sacrificed, and the brains were processed for histochemical assays for β-gal activity. Weiss, column 47, lines 24-32. Weiss stated that: 1) infusion of EGF resulted in an expansion of the population of β-gal labeled cells from 20 cells per brain up to an average of 150 cells per brain, and migration of these cells away from the ventricles; 2) infusion of FGF-2 resulted in an increase in the number of β-gal labeled cells, but this increase was not accompanied by migration; and 3) infusion of EGF and FGF together resulted in an expansion of β-gal labeled cells from 20 cells per brain to an average of 350 cells per brain.

Example 27 does not disclose administration of a TGF-α polypeptide or a functional fragment thereof. In Example 27, there is no CNS damage or lesion. In Example 27, there is no administration outside the ventricles. In Example 27, there is no migration of a neural progenitor cell or progeny thereof to

a site of damage or lesion in the CNS. Thus, Example 27 neither discloses nor suggests the instant invention as claimed.

In discussing Example 27, Weiss states:

> 65 Under normal conditions subependymal precursors do not differentiate or migrate, rather, their fate appears to be cell death after an undefined number of cell divisions (Morshead
>
> **27**
>
> and Van der Kooy, supra). This explanation is also supported by PCR evidence, as described above. Injection of growth factors into the lateral ventricle alters this fate. As described in more detail in Example 27 below, retroviruses were injected into the lateral ventricles for six consecutive days. Implanting cannulae attached to EGF-filled osmotic pumps into the lateral ventricles on the same day as (and 1 or 6 days following) retrovirus injection results in an increase in the total number of RV-β-gal labelled cells 6 days later (from an average of 20 cells/brain to 150 cells/brain).

(emphasis added)

> It is known from the PCR experiments described above that 6 days following retroviral injection no cells exist that contain non-expressed retroviral DNA. Thus these results indicate that the EGF-induced increase in β-gal positive cell number is due to the expansion of the clone size of the retrovirally labelled constitutively proliferative population. It is also possible that part of this increase is due to the activation by EGF of a relatively quiescent stem cell.
>
> Interestingly, this expansion of the number of B-gal labelled cells is accompanied by the migration of these cells away from the subependymal medially, laterally, rostrally, and caudally with subsequent differentiation. Thus, infusion of EGF or similar growth factors induces the proliferation, migration and differentiation of neural stem cells and progenitor cells in vivo, and can be used therapeutically to replace neural cells lost due to injury or disease. In a preferred embodiment EGF and FGF are administered together or sequentially.

Weiss, column 26, line 65 to column 27, line 26.

However, as explained by Dr. Fallon during the in-person interview, the "increase" [e.g., from 20 cells per brain to up to an average of 150 cells per brain, as discussed in Weiss, above] observed by Weiss is on the order of "background noise," in contrast to the therapeutically relevant increase provided by the claimed invention. In fact, as Dr. Fallon explained during the interview, such a background level of increase can be effected merely by inserting a needle into the area.

EXAMPLE 28

In Vivo Proliferation of Neural Stem Cells of the Third and Fourth Ventricles and the Central Canal

A retroviral construct containing the β-galactosidase gene is microinjected (as in Example 27) into the III ventricle of the dienecphalon, IV ventricle of the brain stem and central canal of the spinal cord. Minipumps containing EGF and FGF are then used to continuously administer growth factors for six days (as in Example 27) into the same portion of the ventricular system that the retroviral construct was administered. This produces an increase in the number of β-galactosidase producing cells which survive and migrate out into the tissue near the III ventricle, IV ventricle and central canal of the spinal cord forming new neurons and glia.

(emphasis added)

Example 28 discusses *in vivo* proliferation of neural stem cells of the third and fourth ventricles and the central canal. A retroviral construct containing the β-gal gene was injected as discussed in Example 27 into the third and fourth ventricles and into the central canal.[11] Weiss, column 47, lines 56-59. Minipumps containing EGF and FGF were used to administer growth factors for six days into the same portion of the ventricular system as the β-gal retrovirus. Weiss, column 47, lines 59-63. Weiss stated that the

[11] Weiss defines "ventricle" as "any cavity or passageway within the CNS through which cerebral spinal fluid flows" (which would include the lumbar cistern); furthermore, Weiss states that the term "ventricle" encompasses the lateral, third, and

administration produced an increase in the number of β-gal producing cells that survive and migrate out into the tissue near the third and fourth ventricles and the central canal.

Example 28 does not disclose administration of a TGF-α polypeptide or a functional fragment thereof. In Example 28, there is no CNS damage or lesion. In Example 28, there is no administration outside the ventricles. In Example 28, there is no migration of a neural progenitor cell or progeny thereof to a site of damage or lesion in the CNS. Thus, Example 28 neither discloses nor suggests the instant invention as claimed.

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EXAMPLE 29

In Vivo Modification and Proliferation of Neural Stem Cells and Differentiation of Neural Stem Cell Progeny of the Lateral Ventricle

A retroviral construct containing the TH gene as well as the β-galactosidase gene is microinjected into the adult lateral ventricle as in Example 27. Minipumps containing EGF, FGF, or EGF and FGF together are then used to continuously administer the growth factor(s) into the lateral ventricle for 6 days as in Example 27. As the infected subependymal cells migrate out into the striatum they differentiate into neuronal cells that produce dopamine as measured directly by immunofluorescence with an antibody and (from a direct functional assay) by the ability to overcome the rotational bias produced by unilateral 6-hydroxydopamine lesions.

(emphasis added)

Example 29 discusses *in vivo* modification and proliferation of neural stem cells and differentiation of neural stem cell progeny of the lateral ventricle. A retroviral construct containing the tyrosine hydroxylase (TH) gene and the β-gal gene was injected into the lateral ventricles as discussed in Example 27. Weiss, column 48, lines 6-8. Minipumps containing EGF, FGF, or EGF and FGF together were used to administer the factors into the lateral ventricles for 6 days. Weiss, column 48, lines 8-11. Weiss states that

fourth ventricles, and the central canal, cerebral aqueduct, and other CNS cavities. Weiss, column 13, lines 4-11.

as subependymal cells migrate out into the striatum, they differentiate into neuronal cells that produce dopamine. Weiss, column 48, lines 11-15.

Example 29 does not disclose administration of a TGF-α polypeptide or a functional fragment thereof. In Example 29, there is no CNS damage or lesion. In Example 29, there is no administration outside the ventricles. In Example 29, there is no migration of a neural progenitor cell or progeny thereof to a site of damage or lesion in the CNS. Thus, Example 29 neither discloses nor suggests the instant invention as claimed.

EXAMPLE 30

In Vivo Infusion of Growth Factors into Ventricles to Obtain Elevated Numbers of Neural Stem Cells

Adult male CD_1 albino mice (30–35g) from Charles River were anaesthetized with sodium pentobarbital (0.40 mL of a 10% solution) and placed in a stereotaxic apparatus. The dorsal aspect of the skull was exposed with a longitudinal incision. Cannulas were inserted into the fourth ventricle (stereotaxic coordinates A/P -7.0, L±0.3 D/V -5.8), cerebral aqueduct (A/P -4.8 L±D/V -2.6), or central canal (D/V -1.5). The cannulae were attached with sterile tubing to subcutaneous positioned ALZET osmotic mini-pumps containing 25 μg/mL. EGF (Becton 40001) and/or 25 μg/mL. FGP-2 (R&D Systems 233-FB). Pumps containing sterile saline plus 0.1% mouse albumin (Sigma A3134) were used as controls. The incisions were closed with dental cement.

(emphasis added)

Six days following surgery mice were injected with 0.15 mL BrdU (Sigma B5002); 18 mg/mL in 0.007% NaOH/0.1M PBS) every 2 hours for 8 hours. They were killed 0.5 hours after the last injection with an anaesthetic overdose, and transcardially perfused with 10 mL of ice-cold sterile saline followed by 10mL of ice-cold Bouin's fixative (5% glacial acetic acid, 9% formaldehyde, 70% picric acid). The cervical spinal cord region was dissected out and post-fixed overnight at 4° C. in Bouin's post-fixative solution (9% formaldehyde, 70% picric acid). The following day the tissue was cryoprotected by immersion in 10% sucrose for 2 hours, 20% sucrose for 2 hours, and 30% sucrose overnight. The tissue was frozen in powdered dry ice, mounted in Tissue-Tek (Miles 4583) at −18° C., and 30 μm serial sagittal sections were mounted onto gel-subbed glass slides. Each slide also contained one or more 30 μm coronal sections through the lateral ventricles from the brain of the same animal to serve as a positive control. Slides were kept at −80° C. until processed. Immunohistochemistry: Slides were rinsed in PBS 3×15 minutes in 0.1M PBS at room temperature, hydrolyzed with 1N HCl for 60 minutes at 37° C., rinsed for 3×15 minutes in 0.1M PBS at room temperature, placed in 6% H_2O_2 in methanol for 30 minutes at room temperature, rinsed for 3×15 minutes in 0.1M PBS at room temperature, and incubated in 10% normal horse serum (Sigma H-0146) in 0.1M PBS or 20 minutes at room temperature. Slides were incubated overnight at room temperature in anti-BrdU monoclonal antibody (Becton 7580) that was diluted 1:50 in 0.1M PBS containing 1.5% normal horse serum and 0.3% TRITON®. The following day the slides were rinsed in PBS for 3×10 minutes in 0.1M PBS at room temperature, incubated with biotinylated horse anti-mouse IgG (Vector BA-2000) for 2 hours at room temperature, rinsed for 3×15 minutes in 0.1M PBS at room

temperature, incubated in ABC reagent (Vector PK-6100) for 2 hours at room temperature, rinsed for 3×15 minutes in 0.1M PBS at room temperature, and developed with DAB reagent for 2 to 4 minutes. The slides were coverslipped with Aqua Polymount (Polysciences 18606). The number of BrdU positive cells was counted per cervical spinal cord section. Some BrdU labelled cells were found in the saline control sections. Treatment with either EGF or FGF-2 resulted in a significant increase in the number of BrdU labelled cells seen compared to control. The combination of EGF plus FGF-2 produced even a greater amount of BrdU positive cells per section.

Example 30 discusses *in vivo* infusion of growth factors into ventricles, the cerebral aqueduct, or the central canal to obtain elevated numbers of neural stem cells.[12] EGF, FGF-2, or EGF and FGF-2 were administered into the fourth ventricle using an osmotic mini-pump. Weiss, column 48, lines 23-35. Six days later, mice were injected with BrdU; following which the mice were sacrificed and tissues prepared for histochemistry. Weiss, column 48, lines 36-42. Weiss stated that: 1) treatment with either EGF or FGF-2 resulted in an significant increase in the number of BrdU labeled cells; and 2) the combination of EGF and FGF-2 produced even a greater amount of BrdU positive cells. Weiss, column 49, lines 8-12.

Example 30 does not disclose administration of a TGF-α polypeptide or a functional fragment thereof. In Example 30, there is no CNS damage or lesion. In Example 30, there is no administration outside the ventricles. In Example 30, there is no migration of a neural progenitor cell or progeny thereof to a site of damage or lesion in the CNS. Thus, Example 30 neither discloses nor suggests the instant invention as claimed.

The December 2, 2003 Office Action stated that "the method may be used in areas of demyelination or autoimmune disease such as MS for proliferation of glial schwann, see in particular columns 24-25. December 2, 2003 Office Action, page 5. However, at columns 24-25, Weiss discusses **injection of neural stem cell progeny**. **Administering cells is not administering a TGF-α polypeptide.** Replacing cells to effect a therapy is not the same as inducing migration of endogenous neural progenitor cells or progeny to a site of CNS damage to effect therapy.

The discussion at columns 24-25 is under the section entitled "Transplantation of Neural Stem Cell Progeny Alleviate Disorders of the CNS in Animal Models Caused by Disease or Injury." In this section, Weiss states: "Neural stem cell progeny can be produced and transplanted using the above procedures to treat demyelination diseases." Weiss, column 24, lines 6-8. Weiss further states: "Oligodendrocytes derived

[12] Weiss defines "ventricle" as "any cavity or passageway within the CNS through which cerebral spinal fluid flows" (which would include the lumbar cistern); furthermore, Weiss states that the term "ventricle" encompasses the lateral, third, and

from neural stem cell progeny proliferated and differentiated in vitro may be injected into the demyelinated target areas in the recipient." Weiss, column 24, lines 33-35. Weiss further states: "A preferred treatment of demyelination disease uses undifferentiated neural stem cell progeny." Weiss, column 24, lines 40-41. Weiss further states: "The injection of neural stem cell progeny in remyelination therapy provides, amongst other types of cells, a source of immature type I astrocytes at the implant site." Weiss, column 24, lines 53-55. Finally, Weiss states: "Any suitable method for the implantation of precursor cells near to the demyelinated targets may be used..." Weiss, column 25, lines 9-10. Thus, the discussion in Weiss at columns 24-25 relates only to administration of **cells**.

There is no disclosure or suggestion at Weiss, columns 24-25, of a method for attracting a neural progenitor cell, or a progeny thereof, to a site of damage or lesion in a CNS tissue, the method comprising administering to an individual having CNS damage or lesion a TGF-α polypeptide or functional fragment thereof, wherein the administration is outside the ventricles.

The December 2, 2003 Office Action stated: "The method is also disclosed for use in the replacement of neurons, for example as transplants or grafts, disclosed at column 23." December 2, 2003 Office Action, page 5. However, at column 23, Weiss discusses **injection of neural stem cell progeny**. The discussion at column 23 is under the section entitled "Transplantation of Neural Stem Cell Progeny Alleviate Disorders of the CNS in Animal Models Caused by Disease or Injury." In this section, Weiss states: "Transplantation [of cells] can be done bilaterally, or, in the case of a patient suffering from Parkinson's Disease, contralateral to the most affected side." Weiss, column 23, lines 5-5. Weiss further states: "Cells are delivered throughout any affected neural area, in particular to the basal ganglia, and preferably to the caudate and putamen, the nucleus basalis or the substantia nigra." Weiss, column 23, lines 12-15. Weiss further states: "Neural stem cell progeny when administered to the particular neural region preferably form a neural graft..." Weiss, column 23, lines 33-34. Thus, the discussion in Weiss at column 23 relates only to administration of **cells**.

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fourth ventricles, and the central canal, cerebral aqueduct, and other CNS cavities. Weiss, column 13, lines 4-11.

There is no disclosure or suggestion at Weiss, column 23, of a method for attracting a neural progenitor cell, or a progeny thereof, to a site of damage or lesion in a CNS tissue, the method comprising administering to an individual having CNS damage or lesion a TGF-α polypeptide or functional fragment thereof, wherein the administration is outside the ventricles.

The December 2, 2003 Office Action stated: "Weiss further teaches that these effects may be achieved by direct administration, thus obviating particular problems associate with transplant, see in particular column 12-25 in regard to culture, modification and transplantation of cells and columns 25-29 for the alternative method of direct administration for production of the appropriate cells and treatment in vivo." December 2, 2003 Office Action, page 5. The December 2, 2003 Office Action further stated: "The cells so produced may be generically used to replace damaged or missing neurons and/or glia, see in particular Abstract and column 25, lines 34-41." December 2, 2003 Office Action, page 5.

It is unclear why the Examiner cited portions of the Weiss disclosure relating to administration of cells. The instant method as claimed recites administration of a TGF-α polypeptide or a functional fragment thereof. Administration of **cells** is not equivalent to administration of a TGF-α **polypeptide** or a functional fragment thereof. A disclosure of administration of **cells** does not anticipate a method comprising administration of a TGF-α **polypeptide** or functional fragment thereof.

The December 2, 2003 Office Action stated that Weiss teaches injection of growth factors to animals having CNS damages or lesion; and cited Weiss, column 22, lines 10-17.

However, at column 22, lines 10-17, Weiss states:

> The neural stem cell progeny can be administered to any animal with abnormal neurological or neurodegenerative symptoms obtained in any manner, including those obtained as a result of mechanical, chemical, or electrolytic lesions, as a result of experimental aspiration of neural areas, or as a result of aging processes. Particularly preferable lesions in non-human animal models are obtained with 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP), ibotenic acid and the like.

Thus, at column 22, lines 10-17, Weiss only discusses administration of **cells**.

The December 2, 2003 Office Action stated that Weiss "is not limited to ventricular administration of growth factor." December 2, 2003 Office Action, page 5. The December 2, 2003 Office Action stated that "Weiss teaches administration other than in the ventricle, see in particular oral administration, injection, injection cannula, timed release apparati at the desired site, see in particular column 25, line 20 to column 26, line 15." December 2, 2003 Office Action, page 5.

However, the portion of Weiss cited by the Examiner is in the section entitled "In Vivo Proliferation, Differentiation, and Genetic Modification of Neural Stem Cell Progeny." In this section, as discussed above, for treatment of specific CNS disorders, Weiss discusses administration of growth factors **to the ventricles**. Furthermore, there is no discussion of administration, to an individual having a CNS lesion or damage, of a TGF-α polypeptide or function fragment thereof to a site outside the ventricles, such that **migration** of neural progenitor cells toward the site of CNS damage or lesion is induced.

The December 2, 2003 Office Action stated: "Weiss teaches that administration may be through injection and thus includes administration other than by the digestive tract." Office Action, page 6. The Examiner has apparently confused a site of administration with a mode of administration. The terms "injection, injection cannula, and timed release apparati" are not sites of administration; instead, these are modes of administration. For example, a factor can be administered by injection directly into the ventricles,

or by a timed release apparatus such as an osmotic pump that administers to the ventricles. Weiss, column 26, lines 4-10.

The December 2, 2003 Office Action stated that Weiss teaches that the administration may be "via culture of cells with TGF-alpha, via transplantation of cells maintained or produced under such culture conditions, or via genetic manipulation of cells to provide the growth factor to the host either in vitro or in vivo." December 2, 2003 Office Action, page 6. The December 2, 2003 Office Action cited Weiss at column 10, line 23 to column 11, line 4.

SUMMARY OF THE INVENTION

This invention provides in one aspect a composition for inducing the proliferation of a multipotent neural stem cell comprising a culture medium supplemented with at least one growth factor, preferably epidermal growth factor or transforming growth factor alpha.

At column 10, lines 23-27, Weiss states that the invention provides a composition for inducing proliferation of a multipotent neural stem cell, the composition comprising a culture medium supplemented with a growth factor. At column 10, lines 23-27, the discussion is merely of a composition that induces proliferation *in vitro*. A discussion of inducing *in vitro* proliferation cannot anticipate an *in vivo* method of inducing migration of neural progenitor cells or progeny to a site of CNS lesion or damage. The discussion, at column 10, lines 23-27 of Weiss, does not anticipate a method for attracting a neural progenitor cell, or a progeny thereof, to a site of damage or lesion in a CNS tissue, the method comprising administering to an individual having CNS damage or lesion a TGF-α polypeptide or functional fragment thereof, wherein the administration is outside the ventricles.

The invention also provides a method for the in vitro proliferation and differentiation of neural stem cells and stem cell progeny comprising the steps of (a) isolating the cell from a mammal, (b) exposing the cell to a culture medium containing a growth factor, (c) inducing the cell to proliferate, and (d) inducing the cell to differentiate. Proliferation and perpetuation of the neural stem cell progeny can be carried out either in suspension cultures, or by allowing cells to adhere to a fixed substrate. Proliferation and differentiation can be done before or after transplantation, and in various combinations of in vitro or in vivo conditions, including (1) proliferation and differentiation in vitro, then transplantation, (2) proliferation in vitro, transplantation, then further proliferation and differentiation in vivo, and (3) proliferation in vitro, transplantation and differentiation in vivo.

At column 10, lines 28-43, Weiss discusses a method for the *in vitro* proliferation and differentiation of neural stem cells and stem cell progeny. Such discussion does not anticipate a method for attracting a neural progenitor cell, or a progeny thereof, to a site of damage or lesion in a CNS tissue, the method comprising administering to an individual having CNS damage or lesion a TGF-α polypeptide or functional fragment thereof, wherein the administration is outside the ventricles.

The invention also provides for the proliferation and differentiation of the progenitor cells in vivo, which can be done directly in the host without the need for transplantation.

The invention also provides a method for the in vivo transplantation of neural stem cell progeny, treated as in any of (1) through (3) above, which comprises implanting, into a mammal, these cells which have been treated with at least one growth factor.

Furthermore, the invention provides a method for treating neurodegenerative diseases comprising administering to a mammal neural stem cell progeny which have been treated as in any of (1) through (3), and induced to differentiate into neurons and/or glia.

At column 10, lines 44-46, Weiss states that the invention provides for proliferation and differentiation of the progenitor cells *in vivo*. Such a statement does not anticipate a method for attracting a neural progenitor cell, or a progeny thereof, to a site of damage or lesion in a CNS tissue, the method

comprising administering to an individual having CNS damage or lesion a TGF-α polypeptide or functional fragment thereof, wherein the administration is outside the ventricles.

At column 10, lines 47-51, Weiss states that the invention provides a method for the *in vivo* transplantation of neural stem cell progeny, comprising implanting cells that have been treated with a growth factor into a mammal. Such a statement does not anticipate a method for attracting a neural progenitor cell, or a progeny thereof, to a site of damage or lesion in a CNS tissue, the method comprising administering to an individual having CNS damage or lesion a TGF-α polypeptide or functional fragment thereof, wherein the administration is outside the ventricles.

At column 10, lines 52-56, Weiss states that the invention provides a method for treating neurodegenerative diseases, comprising administering to a mammal neural stem cell progeny. Such a statement does not anticipate a method for attracting a neural progenitor cell, or a progeny thereof, to a site of damage or lesion in a CNS tissue, the method comprising administering to an individual having CNS damage or lesion a TGF-α polypeptide or functional fragment thereof, wherein the administration is outside the ventricles.

The invention also provides a method for treating neurodegenerative disease comprising stimulating in vivo mammalian CNS neural stem cells to proliferate and the neural stem cell progeny to differentiate into neurons and/or glia.

The invention also provides a method for the transfection of neural stem cells and stem cell progeny with vectors which can express the gene products for growth factors, growth factor receptors, and peptide neurotransmitters, or express enzymes which are involved in the synthesis of neurotransmitters, including those for amino acids, biogenic amines and neuropeptides, and for the transplantation of these transfected cells into regions of neurodegeneration.

At column 10, lines 56-59, Weiss states that the invention provides a method for treating neurodegenerative disease comprising stimulating *in vivo* mammalian CNS neural stem cells to proliferate and the neural stem cell progeny to differentiate into neurons and/or glia. Such a statement does not anticipate a method for attracting a neural progenitor cell, or a progeny thereof, to a site of damage or lesion

in a CNS tissue, the method comprising administering to an individual having CNS damage or lesion a TGF-α polypeptide or functional fragment thereof, wherein the administration is outside the ventricles.

At column 10, lines 60-67, Weiss states that the invention provides a method for transfection of neural stem cells and stem cell progeny with vectors that can express the gene products for growth factors, and for the transplantation of the transfected cells into regions of neurodegeneration. Such a statement does not anticipate a method for attracting a neural progenitor cell, or a progeny thereof, to a site of damage or lesion in a CNS tissue, the method comprising administering to an individual having CNS damage or lesion a TGF-α polypeptide or functional fragment thereof, wherein the administration is outside the ventricles.

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In a still further aspect, the invention provides a method for the screening of potential neurologically therapeutic pharmaceuticals using neural stem cell progeny which have been proliferated in vitro.

At column 11, lines 1-4, Weiss states that the invention provides a method for screening of potential neurologically therapeutic pharmaceuticals, using neural stem cell progeny. Such a statement does not anticipate a method for attracting a neural progenitor cell, or a progeny thereof, to a site of damage or lesion in a CNS tissue, the method comprising administering to an individual having CNS damage or lesion a TGF-α polypeptide or functional fragment thereof, wherein the administration is outside the ventricles.

The December 2, 2003 Office Action stated: "Weiss teaches that the direct administration may be so as to provide proliferation and differentiation of the cells as described by the noted genetic modifications, or culture and transplantation methods provided"; and "Such mechanisms and sites of interest include transplantation or delivery to basal ganglia, caudate, putamen, nucleus basalis or substantia nigra, i.e., into the striatum as claimed, see in particular column 23, lines 4-21, column 26, lines 21-26 and 61-64." However, at column 23, lines 4-21, Weiss discusses **transplantation of cells**. A discussion of transplantation of cells into a site in the brain does not anticipate a method involving administering a TGF-α polypeptide or functional fragment thereof. At column 26, lines 15-26, Weiss states explicitly that for the treatment of the recited neurological disorders, growth factors would be delivered **to the ventricles** of the

forebrain. Such does not anticipate the instant methods as claimed, which recite administration to sites **outside the ventricles**. At column 26, lines 61-64, Weiss states that a growth factor can be administered **to the lumbar cistern**.[13]

The December 2, 2003 Office Action stated that for the treatment of spinal cord injury, MS or other demyelinating diseases, "growth factors would be delivered to spinal cord as in Examples 15-17." December 2, 2003 Office Action, page 7. However, as discussed in the following paragraphs, Examples 15-17 relate to transplantation of **cells** into experimental animals. None of Examples 15-17 discloses administration of a TGF-α polypeptide or a functional fragment thereof.

Example 15 discusses transplanting a suspension of cells, which were proliferated *in vitro*, into the spinal cord of myelin deficient rats. Nowhere in Example 15 is there any disclosure whatsoever of administration of a TGF-α polypeptide or a functional fragment thereof.

Example 16 discusses transplanting neural stem cell progeny prepared from human fetal tissue into the spinal cord. Nowhere in Example 16 is there any disclosure whatsoever of administration of a TGF-α polypeptide or a functional fragment thereof.

Example 17 discusses transplanting neural stem cell progeny prepared from human fetal tissue into the spinal cord. Nowhere in Example 17 is there any disclosure whatsoever of administration of a TGF-α polypeptide or a functional fragment thereof.

The December 2, 2003 Office Action stated that Example 44 teaches neural stem cell proliferation in spinal cord tissue, and "column 62 teaches mouse models of spinal cord injury and disease treatment via transplantation into lumbar lateral funiculus." December 2, 2003 Office Action, page 7. The December 2,

[13] Weiss defines "ventricle" as "any cavity or passageway within the CNS through which cerebral spinal fluid flows" (which would include the lumbar cistern); furthermore, Weiss states that the term "ventricle" encompasses the lateral, third, and fourth ventricles, and the central canal, cerebral aqueduct, and other CNS cavities. Weiss, column 13, lines 4-11.

2003 Office Action concluded that Weiss acknowledges administration via various mechanisms outside the ventricles.

However, Example 44 discusses the response of *in vitro* cultured cells, derived from mouse spinal cord tissue, to EGF alone, EGF and bFGF together, or bFGF and heparan together. Nowhere in Example 44 is there any disclosure whatsoever of administration of a TGF-α polypeptide or a functional fragment thereof.

Furthermore, at column 62, Weiss discusses transplanting **cells** into the lumbar lateral funiculus of experimental animals. Nowhere in column 62 of Weiss is there any disclosure whatsoever of administration of a TGF-α polypeptide or a functional fragment thereof.

Of most importance here, when discussing administration of a growth factor in the context of treating a CNS disorder in an individual having a CNS damage or lesion, Weiss states that a growth factor is administered **to the ventricles** (see, *e.g.*, Weiss, column 26, lines 15-26). This is exactly the opposite of what is claimed. Thus, when discussing administration in the context of stimulating migration of a neural progenitor cell or progeny thereof to a site of CNS damage or lesion, Weiss repeatedly instructs administration into the ventricles. Weiss's definition of "ventricle," as noted above, makes it clear that this is the opposite of the claimed invention, which requires administration **outside** the ventricles.

Examples 27-30 of Weiss discuss administration of EGF, FGF, or a combination of EGF and FGF to or into the ventricles. Again, this is opposite of what is claimed.

In the context of treating Huntington's Disease, Parkinson's Disease, and Alzheimer's Disease, Weiss states that "growth factors or other neurological agents would be delivered to the ventricles of the forebrain to affect in vivo modification or manipulation of the stem cells." Weiss, column 26, lines 16-21, emphasis added. In the context of treating Parkinson's Disease, Weiss states that "Dopamine cells can be

generated in the striatum by the administration of a composition comprising growth factors to the lateral ventricle." Weiss, column 26, lines 38-43, emphasis added. Once again, this is opposite of what is claimed.

Weiss discusses genetic modification of neural precursor cell *in vitro* so that the cell secretes a growth factor. Weiss, column 21, lines 1-9. Weiss discusses implantation of genetically modified precursor cells into the CNS of a recipient. Weiss also discusses transplantation of non-transfected cells in a Huntington's Disease animal model, and a Parkinson's Disease animal model. Weiss, column 60, lines 12-67. However, the instant methods as claimed recite administration of a polypeptide, not a cell, genetically modified or otherwise. Administration of a cell is not equivalent to administration of a polypeptide. Thus, this aspect of the disclosure of Weiss is not relevant to the instant claims.

As also discussed during the interview, Dr. Fallon explained that TGF-α administration into the ventricles simply does not provide for a therapeutically significant migration of neural progenitor cells toward a site of CNS injury. As Dr. Fallon explained, there are a number of barriers between the ventricles and the brain tissue outside the ventricles. Neural progenitor cells resident within the ventricles normally migrate through the ventricles toward the olfactory bulb, and do not normally migrate through these barriers into the brain tissue outside the ventricles.

Weiss itself, particularly in Examples 27-30, evidences the effect of this barrier. Intraventricular administration of a growth factor to non-injured animals resulted in meager, background level, and certainly therapeutically insignificant, proliferation (an increase of from about 20 to 350 cells) with non-directed migration (Column 27, lines 17-26). As explained by Dr. Fallon during the interview, the "increase" observed by Weiss is on the order of "background noise" in contrast to the therapeutically relevant increase provided by the claimed invention. Moreover, as evidenced in the instant specification, administration of TGF-α inside the ventricles (i.e., by intracerebroventricular infusion) does not induce migration (as detected by formation of a striatal ridge). Specifically:

> All rats receiving infusions into brain areas other than the striatum received two-week TGFα infusions and nigral 6-OHDA lesions. Intracerebroventricular (ICV) infusion of growth factor ipsilateral to the lesion stimulated the buildup of cells in the adjacent ventricular wall, but did not induce formation of the striatal ridge in any of the animals.
>
> (specification page 57, lines 7-11; emphasis added)

As discussed above, Weiss neither discloses nor suggests a method of attracting a neural progenitor cell, or a progeny of a neural progenitor cell, to a site of CNS damage or lesion, the method comprising administering a growth factor such as TGF-α to an individual having a CNS damage or lesion, wherein administration is outside the ventricles, wherein the neural progenitor cell or progeny thereof migrates to the site of damage or lesion in the CNS. Accordingly, Weiss cannot anticipate the instant invention as claimed.

III. WHETHER THE INVENTION IS OBVIOUS UNDER 35 U.S.C. §103 IN VIEW OF U.S. PATENT NO. 5,980,885

The December 2, 2003 Office Action stated that:

> Requirements for establishing a prima facie case of obviousness
>
> To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *In re Fine*, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Jones,* 21 USPQ2d 1941 (Fed. Cir. 1992). Second, there must be a reasonable expectation of success. *In re Merck & Co., Inc.*, 231 USPQ 375 (Fed. Cir. 1986). Finally, the prior art reference, or references when combined, must teach or suggest all the claim limitations. *In re Roy*ka, 180 USPQ 580 (CCPA 1974). All three criteria must be met. If any one of these three criteria is not met, a *prima facie* case of obviousness has not been established.

The Examiner has not established a prima facie case of obviousness.

1. *There is no suggestion or motivation in Weiss, or in the knowledge generally available to one of ordinary skill in the art, to modify Weiss.*

As discussed above, Weiss repeatedly stresses administration to the ventricles. Weiss states that, for the treatment of CNS disorders, "growth factors or other neurological agents would be delivered **to the ventricles**" (Weiss, column 26, lines 16-20, emphasis added); Weiss states that to modify stem cells *in vivo*, a composition comprising a growth factor is administered "**to the ventricle**" (Weiss, column 26, lines 4-8, emphasis added); Weiss states that a composition comprising a growth factor "may be injected **directly to the ventricles**" (Weiss, column 26, lines 9-10, emphasis added); Weiss states that, for the treatment of Parkinson's disease, dopamine cells can be generated by the "administration of a composition comprising growth factors **to the lateral ventricle**" (Weiss, column 26, lines 42-44, emphasis added); Weiss states that for the treatment of MS and other demyelinating or hypomyelinating disorders, and for the treatment of Amyotrophic Lateral Sclerosis or other motor neuron diseases, "growth factors or other neurological agents would be delivered **to the central canal**" (Weiss, column 26, lines 57-61, emphasis added).[14]

Thus, there is no suggestion in Weiss to modify the discussion of administration of growth factors to the ventricles. The entire emphasis of Weiss is focused on administration of cells to the brain; and the only discussion of administration of growth factors is to the ventricles.

Furthermore, in Examples 27-30, Weiss discusses administration of the growth factors EGF, FGF-2, or a combination of EGF and FGF-2 **into the ventricles** of the forebrain. In Example 27, Weiss states that infusion of EGF into the ventricles resulted in an expansion of β-gal-labeled cells from an average of 20 cells per brain up to an average of 150 cells per brain and the migration of these cells away from the lining of the lateral ventricles. Weiss, column 47, lines 33-38. Weiss gives no indication that such an expansion is

[14] Weiss defines "ventricle" as "any cavity or passageway within the CNS through which cerebral spinal fluid flows" (which would include the lumbar cistern); furthermore, Weiss states that the term "ventricle" encompasses the lateral, third, and

actually within background levels. Thus, from a reading of Examples 27-30, one skilled in the art would have no motivation to administer a growth factor to a site other than the ventricles.

2. *There is no reasonable expectation of success in Weiss.*

Because Weiss does not teach or suggest administration of a growth factor to an individual having a CNS damage or lesion, wherein administration is to a site outside the ventricles, Weiss does not provide a reasonable expectation of success. Those skilled in the art, from reading Weiss, would not have expected that administration to a site other than the ventricles would provide for migration of neural progenitor cells away from the ventricles.

3. *Weiss does not teach or suggest all the claim limitations.*

As discussed amply above, Weiss fails to teach or suggest a method for attracting a neural progenitor cell or progeny thereof to a site of damage or lesion in a CNS tissue. Weiss fails to teach or suggest a method that requires both the presence of a CNS damage or lesion and administration of a TGF-α polypeptide or a functional fragment thereof. Weiss fails to teach or suggest a method that results in migration of neural progenitor cells or their progeny away from the ventricles and toward a site of CNS damage or lesion. Weiss fails to teach or suggest a method that involves administration of a e TGF-α polypeptide (or functional fragment thereof) to a site outside the ventricles.

The Examiner has not considered the teachings of Weiss as a whole.

The analysis presented in the December 2, 2003 Office Action is a hindsight reconstruction of the invention claimed in this application from particular elements of Weiss, taken out of context. The courts have said that this type of analysis is prohibited. Hindsight is not a justifiable basis on which to find that ultimate achievement of a long sought and difficult scientific goal was obvious. *Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.*, 18 USPQ2d 1016 (Fed. Cir. 1991), cert. denied, 502 U.S. 856 (1991).

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fourth ventricles, and the central canal, cerebral aqueduct, and other CNS cavities. Weiss, column 13, lines 4-11.

Specifically, a prior art reference must be read in its entirety for all that it teaches. The courts prohibit hindsight reconstruction of a different invention by cherry-picking particular elements of the prior art reference, and making them work together in a manner that is different from what the reference intends.

It is insufficient that the prior art disclosed the components of a claimed invention, either separately or used in other combinations; there must be some teaching, suggestion, or incentive to make the combination made by the inventor. *Northern Telecom, Inc. v. Datapoint Corp.* 15 USPQ2d 1321 (Fed. Cir. 1990), cert. denied, 498 U.S. 920 (1990). Determination of obviousness can not be based on the hindsight combination of components selectively culled from the prior art to fit the parameters of the claimed invention. *ATD Corp. v. Lydall, Inc.*, 48 USPQ2d 1321 (Fed. Cir. 1998).

The December 2, 2003 Office Action stated that Weiss "renders obvious administration of the growth factors outside the ventricles because the reference teaches the relevant sites outside the ventricles that are to be treated by the neural precursor cells and that are stimulated to proliferate, differentiate and migrate via TGF-alpha exposure" and that Weiss "further teaches direct administration via oral administration, injection and injection cannula." December 2, 2003 Office Action, page 14. However, as discussed above, administration of cells is not equivalent to administration of a TGF-α polypeptide or functional fragment thereof.

Furthermore, *in the context of treating neurological disorders*, Weiss specifically directs one to administer an agent to the ventricles. The only mention in Weiss of oral administration is in the context of increasing cell numbers, not for inducing cell migration in the treatment of a neurological disorder. Finally, as discussed above, injection and injection cannula are not sites of administration; instead, these terms described modes of administration. The only discussion in Weiss of injection is in the context of directly into the ventricles.

The cited statements in Weiss cannot be viewed in the abstract. Rather, they must be considered in the context of the teaching of the entire reference. Further, a rejection cannot be predicated on the mere

indication in Weiss of individual components of claimed limitations. Rather, particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed. *In re Kotzab*, 54 USPQ2d 1308 (Fed. Cir. 2000).

The Examiner has applied the wrong standard for determining obviousness.

The December 2, 2003 Office Action stated that the teachings of Weiss "apparently arise [sic] to that of anticipation." December 2, 2003 Office Action, page 9. However, as noted above, Weiss does not anticipate the invention as claimed. Furthermore, the rejection is under 35 U.S.C.§103, not 35 U.S.C.§102. The standard under 35 U.S.C.§103 is whether the claimed invention is obvious in view of the cited art, not whether the claimed invention is anticipated by the cited art.

The December 2, 2003 Office Action stated that the teachings of Weiss apparently rise to that of anticipation "as the reference is enabling for the artisan for practice of the claimed invention." December 2, 2003 Office Action. While a reference must be enabling in order to anticipate a claimed invention, whether a reference is enabling is not the standard for determining obviousness under 35 U.S.C.§103. As such, the Examiner has applied an incorrect standard for obviousness.

SUMMARY

Conclusion as to the rejection under 35 U.S.C.§112, second paragraph

The rejection under 35 U.S.C.§112, second paragraph, has been adequately addressed by the amendments to claims 2, 3, and 5, shown below, and by cancellation of claim 20 without prejudice to renewal.

Conclusion as to the rejection under 35 U.S.C.§102(e)

Weiss neither discloses nor suggests the instant invention as claimed. Weiss fails to teach a method for attracting a neural progenitor cell or progeny thereof to a site of damage or lesion in a CNS tissue. Weiss fails to teach a method that requires both the presence of a CNS damage or lesion and administration of a TGF-α polypeptide or a functional fragment thereof. Weiss fails to teach a method that results in migration

of neural progenitor cells or their progeny away from the ventricles and toward a site of CNS damage or lesion. Weiss fails to teach a method that involves administration of a e TGF-α polypeptide (or functional fragment thereof) to a site outside the ventricles. Because Weiss fails to disclose each and every element of the instant invention as claimed, Weiss cannot anticipate the instant invention as claimed.

Conclusion as to the rejection under 35 U.S.C.§103(a)

Weiss does not render the instant claims obvious. Weiss does not provide motivation to modify the teachings of Weiss to provide for administration of a TGF-α polypeptide or functional fragment thereof to a site outside the ventricles in an individual having a CNS damage or lesion; Weiss does not provide a reasonable expectation of success that administration of a TGF-α polypeptide or functional fragment thereof to a site outside the ventricles in an individual having a CNS damage or lesion will effect migration of a neural progenitor cell or progeny thereof away from the ventricles and toward the site of CNS damage or lesion; and Weiss does not teach or suggest all of the claim limitations. As such, the Examiner has not established a prima facie case of obviousness, and therefore the claims are not rendered obvious by Weiss.

RELIEF REQUESTED

Appellants respectfully request that the rejection of claims 2, 3, and 5 under 35 U.S.C. §112, second paragraph, the rejection of claims 1-3, 5-8, 33, 63, and 64 under 35 U.S.C. §102(e), and the rejection of claims 1-3, 5-8, 33, 63, and 64 under 35 U.S.C. §103 be reversed, and that the application be remanded to the Examiner with instructions to issue a Notice of Allowance.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: Mar. 9, 2004

By: _____
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APPENDIX OF APPEALED CLAIMS

1. A method for attracting a neural progenitor cell, or a progeny of a neural progenitor cell, to a site of damage or lesion in a central nervous system (CNS) tissue, the method comprising

parenterally administering to an individual having CNS damage or lesion a sufficient amount of a TGF-α polypeptide or a functional fragment thereof, wherein said administration is outside of the ventricles, and wherein said administering effects migration of the neural progenitor cell or progeny thereof to the site of damage or lesion in the CNS tissue.

2. (Currently amended) The method of claim 1, further comprising administering a sufficient amount of the ~~compound~~ TGF-α polypeptide or functional fragment thereof to stimulate differentiation of the neural progenitor cell or progeny thereof.

3. (Currently amended) The method of claim 1, wherein the ~~compound~~ TGF-α polypeptide or functional fragment thereof is administered *in vivo*.

5. (Currently amended) The method of claim 1, wherein the ~~compound~~ TGF-α polypeptide or functional fragment thereof is administered by intrastriatal infusion.

6. The method of claim 1, wherein the central nervous system (CNS) tissue is brain tissue.

7. The method of claim 6, wherein the brain tissue is adjacent to a subependymal zone.

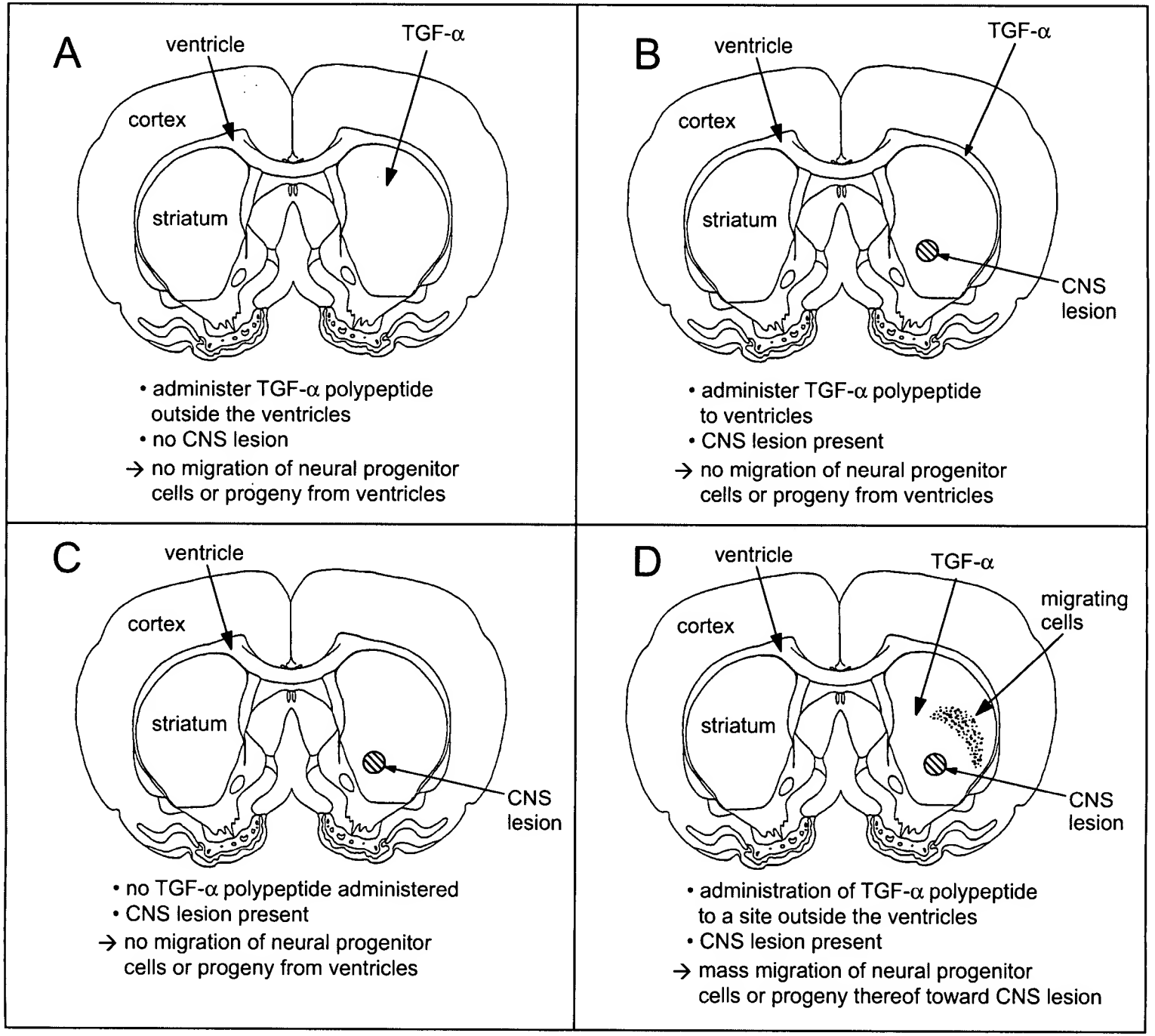
8. The method of claim 1, wherein the central nervous system (CNS) tissue is spinal cord tissue and spinal nerve root origins.

20. (Canceled)

33. A method for attracting a neural progenitor cell, or a progeny thereof, to a site of damage or lesion in a central nervous system (CNS) tissue, the method comprising administering a sufficient amount of transforming growth factor alpha (TGFα) polypeptide, or functional fragment thereof, to the site to attract the neural progenitor cell or its progeny to the site, wherein said administration is outside of the ventricles.

63. A method for attracting a neural progenitor cell, or a progeny thereof, to a site of damage or lesion in a central nervous system (CNS) tissue, the method comprising intrastriatal administering a sufficient amount of transforming growth factor alpha (TGFα) polypeptide, or functional fragment thereof, to the site to attract the neural progenitor cell or its progeny to the site.

64. The method of claim 33, wherein said administration is by continuous infusion.


A
ventricle
TGF-α
cortex
striatum
• administer TGF-α polypeptide outside the ventricles
• no CNS lesion
→ no migration of neural progenitor cells or progeny from ventricles
B
ventricle
TGF-α
cortex
striatum
CNS lesion
• administer TGF-α polypeptide to ventricles
• CNS lesion present
→ no migration of neural progenitor cells or progeny from ventricles
C
ventricle
cortex
striatum
CNS lesion
• no TGF-α polypeptide administered
• CNS lesion present
→ no migration of neural progenitor cells or progeny from ventricles
D
ventricle
TGF-α
migrating cells
cortex
striatum
CNS lesion
• administration of TGF-α polypeptide to a site outside the ventricles
• CNS lesion present
→ mass migration of neural progenitor cells or progeny thereof toward CNS lesion

In vivo induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain

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The development of an *in vivo* procedure for the induction of massive proliferation, directed migration, and neurodifferentiation (PMD) in the damaged adult central nervous system would hold promise for the treatment of human neurodegenerative disorders such as Parkinson's disease. We investigated the *in vivo* induction of PMD in the forebrain of the adult rat by using a combination of 6-hydroxydopamine lesion of the substantia nigra dopaminergic neurons and infusions of transforming growth factor α (TGFα) into forebrain structures. Only in animals with both lesion and infusion of TGFα was there a rapid proliferation of forebrain stem cells followed by a timed migration of a ridge of neuronal and glial progenitors directed toward the region of the TGFα infusion site. Subsequently, increasing numbers of differentiated neurons were observed in the striatum. In behavioral experiments, there was a significant reduction of apomorphine-induced rotations in animals receiving the TGFα infusions. These results show that the brain contains stem cells capable of PMD in response to an exogenously administered growth factor. This finding has significant implications with respect to the development of treatments for both acute neural trauma and neurodegenerative diseases.

Neurogenesis in the adult mammalian central nervous system has been demonstrated in the dentate gyrus of the hippocampus and in the olfactory bulbs of adult birds and rodents, and it has been shown that these cells arise from a rostral migratory stream originating in the subventricular zone (SVZ) (1–3). The SVZ is rich in pluripotent stem cells (4–7), and such cells have also been demonstrated to exist in varying degrees throughout the neuraxis (8–10). While it has been shown that these cells can proliferate *in vitro* in response to extracellular signals and growth factors such as fibroblast growth factor, basic fibroblast growth factor, and ligands that bind the type I family of tyrosine kinase receptors, including epidermal growth factor (EGF) and transforming growth factor α (TGFα) (11–13), *in vivo* studies of proliferation, migration, and differentiation (PMD) have met with only limited success (14, 15). Successful demonstration of *in vivo* PMD in neural tissue would provide tools to begin to develop drugs to treat neurodegenerative diseases such as Parkinson's disease.

Recent evidence suggests that a combination of extracellular signals and microenvironmental conditions may be necessary for the *in vivo* stimulation of neuroepithelial stem cells, including extracellular matrix molecules, paracrine and juxtacrine cell–cell signaling, and growth factor delivery and concentration (16). Magavi *et al.* (17) have demonstrated local *in vivo* neurogenesis in adult mice in response to microenvironmental modification by targeted apoptosis. However, to date, no studies have demonstrated the induction of the massive proliferation, directed migration, and differentiation of endogenous neuroepithelial stem cells into neurons in the broader regions of the nervous system, which would allow repopulation of, and regeneration of, damaged neural tissue. In the present study, we demonstrate the *in vivo* induction of massive PMD in the forebrain of the adult rat following unilateral 6-hydroxydopamine (6-OHDA) lesioning of the substantia nigra dopaminergic neurons and infusions of TGFα into ipsilateral forebrain structures.

Materials and Methods

Adult male Sprague–Dawley albino rats (240–350 g) (n = 130) were obtained from Simonsen Laboratories (Gilroy, CA). The animals were maintained in a temperature- and humidity-controlled campus vivarium. All aspects of animal handling and surgery were in accordance with current National Institutes of Health guidelines and University of California at Irvine Institutional Animal Care and Use Committee protocols. 6-OHDA and TGFα were administered as follows.

6-OHDA Lesions. Animals were anesthetized with i.p. xylazine (8 $mg \cdot kg^{-1}$) and ketamine (100 $mg \cdot kg^{-1}$) (Western Medical Supply, Arcadia, CA). A chilled sterile solution of 1.0–4.8 mg/ml of 6-OHDA (Sigma) in 0.9% saline with 0.01% ascorbic acid was prepared immediately before injection. The animals were immobilized in a Kopf stereotaxic device and placed on a warm mat at 37°C. Using aseptic techniques, 2.5–8 μl of 6-OHDA solution was stereotaxically injected at the rostral border of the substantia nigra–ventral tegmental area (SN-VTA) (+3.7 A/P; +2.1 M/L; +2.0 D/V) at a rate of 1 $\mu l \cdot min^{-1}$ using interaural zero as a reference. The total duration of surgery was approximately 45 min.

Growth Factor Infusions. Osmotic minipumps (models 2002 and 2004, Alzet) were used for TGFα and artificial cerebrospinal fluid (aCSF) infusions. They were implanted at a predetermined period contemporaneous with, or after, the 6-OHDA lesion. The minipumps were filled with approximately 200 μl of a solution containing 10, 50, or 100 μg of TGFα (Stem Cell Pharmaceuticals) in aCSF for experimental animals or plain aCSF for control animals and incubated overnight in normal saline at 37°C before implantation. Under aseptic conditions, the 5-mm cannula attached to the minipump (Brain Infusion Kit, Alzet) was stereotaxically implanted into the left caudate–putamen (+1.2

Abbreviations: PMD, massive proliferation, directed migration, and neurodifferentiation; TGFα, transforming growth factor α; SVZ, subventricular zone; EGF, epidermal growth factor; EGFr, epidermal growth factor receptor family; 6-OHDA, 6-hydroxydopamine; aCSF, artificial cerebrospinal fluid; TH, tyrosine hydroxylase; SN-VTA, substantia nigra–ventral tegmental area; DAT, dopamine transporter.

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A/P; +2.7 M/L) using bregma as a reference. The minipump was placed s.c. in the interscapular region. The infusate was delivered directly into the striatum by the minipump at a rate of approximately 0.5 $\mu l \cdot h^{-1}$. The animals (n = 130) were subdivided into groups receiving simultaneous infusions (TGFα, n = 25; aCSF, n = 13), infusions 2 weeks after 6-OHDA (TGFα, n = 20; aCSF, n = 11), controls receiving infusions without a 6-OHDA lesion (TGFα, n = 8; aCSF, n = 8), intraventricular infusion with 6-OHDA lesion (n = 2), or 6-OHDA lesion without any infusion (n = 43).

Behavior Testing. The apomorphine-induced rotation technique was used to test behavioral changes in both the 6-OHDA lesioned and nonlesioned animals. A fresh solution of apomorphine (0.25 mg/ml) was prepared each time. The animal was set on a rotometer bowl and its behavior was observed for 5 min. It then received an s.c. injection of apomorphine (2.5 mg/kg body weight) and was placed back in its cage and allowed to rest for 10 min. Apomorphine-induced rotations were thereafter observed at 10-min intervals, each session lasting 5 min for a total of 15 min.

***In Situ* Hybridization Histochemistry.** TGFα mRNA probes were generated from a 550-nt *Xba*I–*Bam*HI cDNA fragment from the 5′ end of rat TGFα, subcloned into pGEM 7Zf (Promega). Antisense and sense probes were transcribed with SP6 and T7 polymerases, respectively. Rat EGF receptor (EGFr) mRNA probes were produced from a 718-bp *Bam*HI–*Sph*I insert from the 5′ end of the gene in pGEM 7Zf. Probes for rat tyrosine hydroxylase (TH) were created by using the 1.3-kDa *Bam*HI–*Eco*RI fragment subcloned into pGEM 7Zf. Antisense subclones for EGF receptor and TH were transcribed with T7 polymerase. Sense subclones for EGF receptor and TH were transcribed with SP6 polymerase. All probes were radiolabeled by transcription in the presence of ^{35}S-labeled UTP (NEN).

In situ nucleic acid hybridization was performed according to Simmons *et al.* (44). Parallel sections from experimental and control animals were hybridized overnight at 65°C with sense or antisense probes at a concentration of 107 cpm/ml. Adjacent sections from the same animals were hybridized to each of the probes so that direct comparison could be made of their anatomical distributions.

Slides from experimental and control animals were grouped together and apposed with ^{14}C-labeled brain paste standards to autoradiographic Beta Max Hyperfilm (Amersham Pharmacia) for 3–7 days. After successful development of the autoradiography film, analysis and quantitation was done by using MCID (Imaging Research, St. Catherine's, ON, Canada). Densitometry readings were sampled at multiple sites within each anatomical region of interest and averaged. Relative concentrations of TGFα and EGF receptor from the hybridization process were then estimated by using a computer-generated third-degree polynomial standard curve constructed from the ^{14}C brain paste standards. The estimated values for each region in each treatment group were then averaged and their standard errors were calculated. Brain regions ipsilateral to the experimental treatments were compared with the corresponding regions in control brains at approximately the same positions. Significance of the comparisons was determined by using the Student t test.

BrdUrd Administration. Animals received 50 mg/kg BrdUrd (Boehringer Mannheim) i.p. hourly for 3 days from the day of surgery.

Tissue Preparation. Animals were killed at timed intervals between 1 and 28 days by either decapitation or perfusion. After decapitation, the brains were quickly removed and frozen in isopentane at −20°C. Coronal cryostat sections were cut at 40 μm and thaw adhered to Vectabond (Vector Laboratories)-coated slides. The sections were postfixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h, rinsed in phosphate buffer and air dried, then stored at −20°C until processed.

After cardiac perfusion first with saline then 4% paraformaldehyde, the brains were quickly removed and postfixed in 4% paraformaldehyde for 1 h, then cryoprotected in 30% sucrose overnight at 4°C. The brains were then cooled and cut at −20°C on a freezing microtome.

Immunohistochemistry. Perfused brain tissue was cut on a freezing microtome at 40 μm, and free-floating sections were placed in 0.1 M PBS, pH 7.4. Blocking was done by using 10% normal goat serum with 0.4% Triton X in PBS for 1 h before primary antibody incubation. Primary antibodies were dissolved in blocking solution as follows. Rabbit anti-glial fibrillary acidic protein (1:400, Dako), mouse anti-β-III tubulin (1:100, R & D Systems), rabbit anti-β-III tubulin (1:10,000, R & D Systems), rabbit anti-tyrosine hydroxylase (1:1,000, Sigma), rabbit anti-s100β (1:2,500, R & D Systems), mouse anti-nestin (1:20, Hybridoma Bank), mouse anti-doublecortin (1:250, kind gift of C. Walsh), and rat anti-dopamine transporter (DAT; Chemicon, 1:5,000). The tissues were then incubated overnight at room temperature in primary antibody and then rinsed in PBS and incubated in biotinylated or fluorescent secondary antibodies (anti-mouse, anti-rabbit, anti-rat) in blocking solution. Biotinylated primaries were visualized by using ABC solution (Vector ABC Elite) for 1 h, followed by diaminobenzidine-peroxidase histochemistry (Sigma).

For BrdUrd immunocytochemistry, DNA was denatured by using 50% formamide in 2× SSC for 2 h at 65°C, and tissue sections were then rinsed in 2× SSC for 5 min and incubated in 2 M HCl at 37°C for 30 min. The tissue slices were then rinsed in 0.1 M boric acid, pH 8.5, for 10 min, followed by a 5-min rinse in PBS. For double labeling, tissues were incubated with anti-BrdUrd and either anti-TH or anti-DAT primary antibodies, then processed for fluorescence as above.

Silver Staining for Cellular Morphology. Cells were labeled by using a modification to the Nauta method, similar to procedure 1 of Fink-Heimer (18). Free-floating sections were placed into 0.05% potassium permanganate before treatment with fresh 1% hydroquinone/1% oxalic acid and were then treated with successive uranyl nitrate/silver nitrate solutions of increasing concentration. After another rinse, the sections were reacted in ammoniacal silver, then in ethanol/citric acid/paraformaldehyde reducer, and finally in sodium thiosulfate. After staining, sections were mounted on glass slides, dehydrated, and cover-slipped.

Results

Morphological Studies of PMD Induction. We first determined conditions sufficient to induce the massive proliferation of multipotential stem cells originating in the SVZ and the subsequent directed migration of these neuroprogenitor cells into the striatum. We used combinations of infusions of TGFα into the ipsilateral striatum, and 6-OHDA injections into the ipsilateral SN-VTA (Fig. 1). Because TGFα-responsive stem and neuroprogenitor cells in the SVZ have been shown to express EGFr mRNA, we used *in situ* nucleic acid hybridization and other anatomical and behavioral techniques to demonstrate TGFα responsiveness in the SVZ. In animals receiving 6-OHDA lesions of SN-VTA and aCSF control infusions into the striatum, there was no significant change in the expression of EGFr mRNA in the SVZ (Fig. 1*a*). In a second control group receiving TGFα infusions, but no 6-OHDA lesions, there was a significant increase in the expression of EGFr mRNA in the SVZ for the duration of the infusion (Fig. 1*b*). In the experimental group

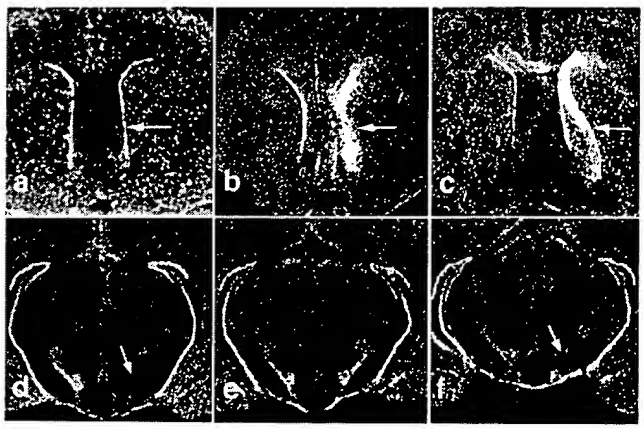

Fig. 1. Autoradiograms of coronal sections of forebrain (*a–c*) and midbrain (*d* and *e*) showing EGFr mRNA expression in the SVZ (arrows in *a–c*) and SN-VTA (*d–f*), respectively. Animals received 6-OHDA lesion of SN-VTA and aCSF infusion into striatum of right side of brain (*a* and *d*), TGFα infusion into striatum but no lesion of SN-VTA (*b* and *e*), or both a 6-OHDA lesion of SN-VTA and a TGFα infusion into striatum (*c* and *f*). There is a significantly higher (200%, $P < 0.01$) expression of EGFr mRNA in the SVZ of animals receiving TGFα (*b* and *c*). When both a 6-OHDA lesion and TGFα infusion are made, an EGFr-mRNA positive ridge lateral to the SVZ is present in the striatum (*c*). The density of EGFr-mRNA in nonridge areas of striatum was unchanged after TGFα infusions ($P < 0.001$). Arrows in *d* and *f* point to the right substantia nigra. The lack of an EGFr *in situ* signal at these sites confirms that the 6-OHDA injection on the right side lesioned the majority of EGFr-expressing dopaminergic neurons in this region.

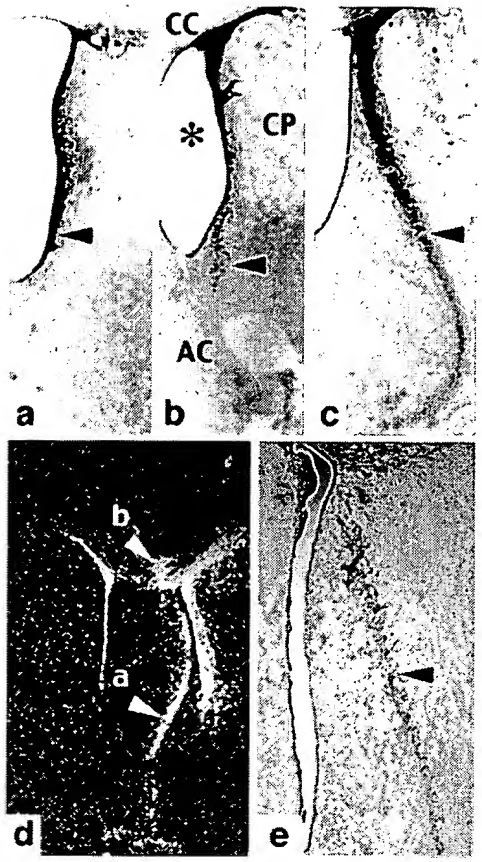

Fig. 2. Time-course analysis of SVZ proliferation and progression of ridge migration. There is an initial proliferation of SVZ cells (arrowhead in *a*) in the first week of TGFα infusion into the caudate-putamen (CP) (*a*), followed by an en masse migration of the ridge into the striatum (arrowhead), starting at 9 days of TGFα infusion (*b*), progressing to the midstriatum by the 14th day (AC, anterior commissure) (*c*) *, Lateral ventricle. Septal infusions resulted in a medially directed EGFr-mRNA positive "septal" ridge (*d*, arrowhead a) originating in the medial SVZ adjacent to the striatum (*d*). Several other migration patterns can be seen, for example a colossal ridge (*d*, arrowhead b) or in the septum of the contralateral medial SVZ after striatal TGFα infusions (*e*).

receiving both a 6-OHDA lesion and a TGFα infusion, there was also a significant increase of EGFr mRNA expression in the SVZ. In animals that received more than 9 days of TGFα infusion, there was also an additional ridge (or thick-layered sheet in three dimensions) of EGFr mRNA-positive cells encroaching into the striatum from the SVZ (Fig. 1*c*). This ridge was most pronounced in animals that received concurrent 6-OHDA lesion and 10, 50, or 100 μg of total TGFα infusion, but also was present in animals receiving TGFα infusions starting 14 days after the 6-OHDA lesion. Both the 50- and 100-μg dose appeared to be equally effective, and the 10-μg dose was minimally less effective in inducing ridge formation.

In a serial time-course analysis, after 4–6 days (Fig. 2*a*) of continuous TGFα infusion in lesioned animals, there was a pronounced thickening of the SVZ. By 9 days (Fig. 2*b*), however, the ridge separates en masse from the SVZ, as well as at later time periods from 14 to 21 days, appearing progressively lateral to the SVZ toward the site of the TGFα infusion (Fig. 2*c*). The exact shape and apparent movement of the ridge depends on the site of the TGFα infusion. For example, a ventral striatal infusion results in an S-shaped ridge (Fig. 2*c*), whereas a dorsal striatal infusion resulted in a shorter and thicker ridge mass adjacent to the dorsal SVZ. The ridge of cells is observed whether the TGFα infusion is started concurrent with the lesion 2 days before or 14 days after the lesion. To determine whether this directed migration is limited to striatal targets, infusions of TGFα were made into the septum. In these cases, there was a massive proliferation of the SVZ, and a subsequent ridge appeared medially in the septum toward the TGFα infusion site (Fig. 2*d*). Additionally, in some cases a smaller ridge of cells could also be seen on the medial side of the contralateral SVZ in the septum (Fig. 2*e*) or migrating through the corpus callosum (Fig. 2*d*). In the cases where there was an infusion of TGFα into the lateral ventricle in 6-OHDA-lesioned animals, there was a temporary proliferation of SVZ cells but no migratory ridge.

Characterization of the Ridge. To further characterize the cells in the ridge, we used a series of morphological and immunocytochemical techniques (Fig. 3). Silver staining revealed fusiform cells in the ridge, oriented orthogonal to the most adjacent area of the SVZ. This is consistent with the possibility that these cells are migrating from the SVZ in parallel (Fig. 3*a*). After TGFα infusion into the brain, systemically administered 5′BrdUrd was incorporated into the SVZ with a specific and massively increased incorporation in the first 3 days. Subsequently, 5′-BrdUrd positive cells were seen in the ridge, striatum, external capsule, and cortex adjacent to the infusion cannula (Figs. 3*b* and 4*a*), indicating that the ridge cells were recently generated de *novo*. To determine the lineage and differentiation of the cells in and around the ridge, we first stained for nestin, a cytoskeletal marker for early lineage neuronal and glial progenitors. The SVZ and ridge cells were nestin-positive from the fourth day of TGFα infusion (Fig. 3*c*). To determine whether the ridge cells are of restricted glial and/or neuronal lineages, we stained for s100β (astrocytic lineage) and β-III tubulin (neuronal lineage). At 1, 4, 7, and 9 days (data not shown) of TGFα infusion, there was no evidence of significant positive staining of these markers, but starting at 14 days, β-III tubulin positive cells were seen (Figs. 3*d* and 4*b*). s100β positive cells were also seen in and

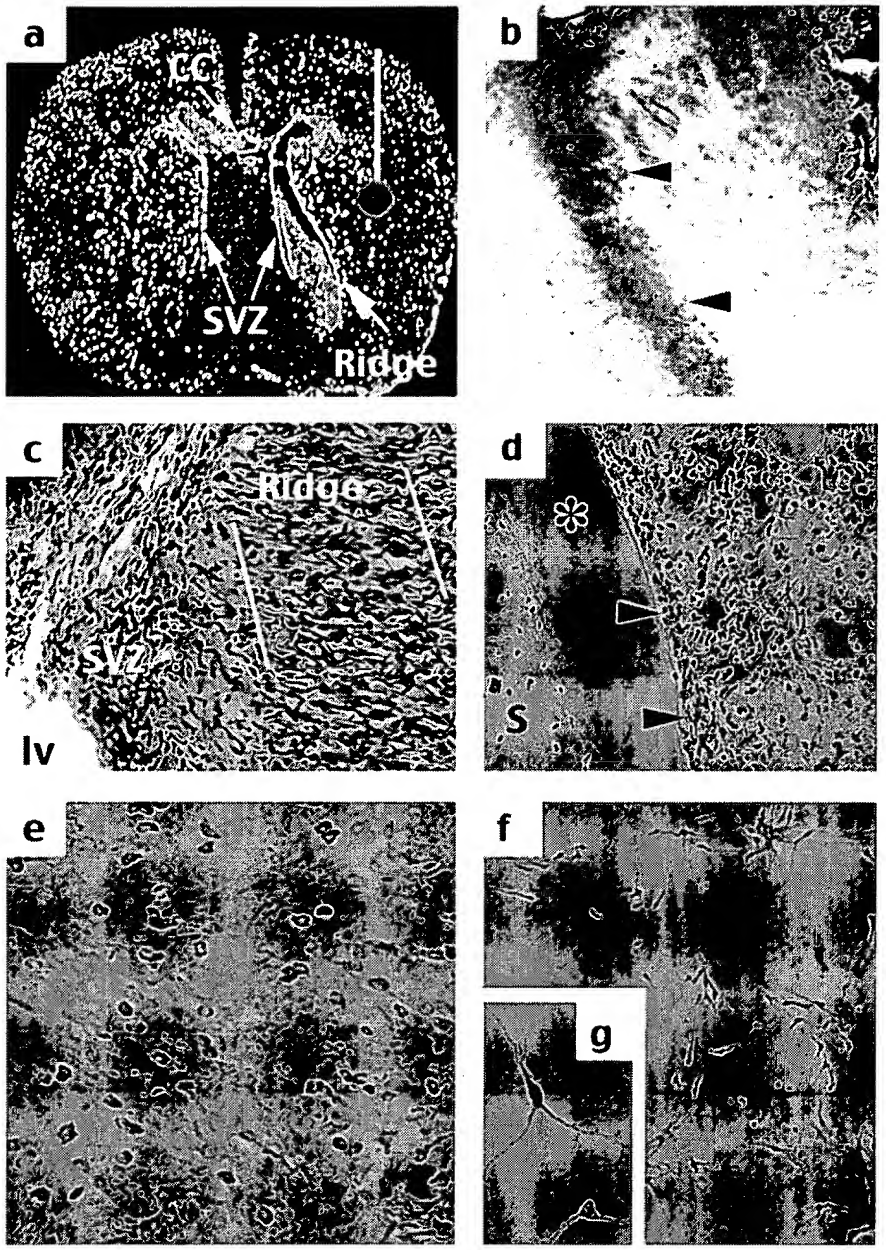

Fig. 3. Further characterization of the TGFα-induced striatal ridge cells. (*a*) Cross section of pseudocolor autoradiographic image (EGFr-mRNA) showing SVZ, location of a ridge, a smaller ridge in the corpus callosum (CC), and a cartoon image of the TGFα infusion cannula (white line) and infusion site in the right caudate putamen (pink circle). (*b*) The ridge cells (arrows) are nestin-positive, showing that they are neural progenitors. (*c*) Silver staining shows a fusiform morphology of the cells in the ridge (outlined by white lines), suggestive of outward migration from the SVZ lining the lateral ventricle (lv). (*d*) BrdUrd was incorporated by SVZ (arrows) and ridge cells laterally in the striatum, but not in the septum (S) after a striatal TGFα infusion. (*e*) Some migrating cells subsequently stained positive for β-III tubulin, a marker for neuronal restricted lineage. (*f*) Longer TGFα infusion times revealed increasing numbers of TH-positive neurons (higher magnification in *g*).

around the ridge (data not shown). Immunofluorescence staining was also carried out on adjacent tissue sections to verify that the ridge is densely populated with BrdUrd-positive cells (Fig. 4*a*) and that large clusters of β-III tubulin-positive neuronal precursors are present in the BrdUrd-rich ridge (Fig. 4 *b* and *c*). Doublecortin, a marker for migrating young neurons, is also present in the ridge cells (Fig. 4 *d* and *e*).

Staining for these restricted astrocytic and neuronal lineage markers was, however, greatly reduced by 28 days. To determine whether TGFα infusions in these same 6-OHDA-lesioned animals result in the new appearance of differentiated neurons in the striatum, TH and DAT immunoreactivity was carried out. Within the second to third week of infusion, occasional TH- and DAT-positive cells were seen near the infusion cannula. From the third and fourth week of infusion, increasing numbers of TH-positive (Fig. 3 *f* and *g*) and DAT-positive (Fig. 4 *f* and *h*) cells were seen in the striatum. In double-labeling experiments, some newly generated (BrdUrd-positive) neurons were also DAT-positive (Fig. 4 *f–h*) or TH-positive (data not shown).

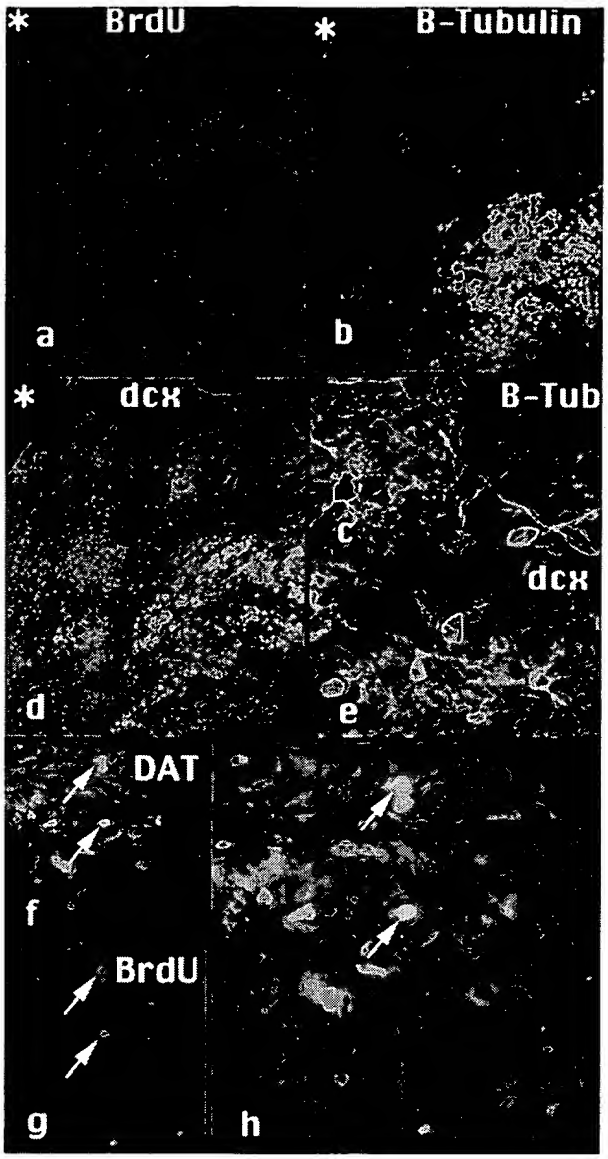

Fig. 4. Further characterization of migratory ridge cells by using fluorescence immunohistochemistry for neuronal markers. There is dense positive staining for BrdUrd (*a*), β-tubulin (*b* and *c*), and doublecortin (*d* and *e*). Labeling of the same section for DAT (*f*), and BrdUrd (*g*) reveals that some neurons are double-labeled for both markers (*h*). White arrows on *f–h* point to double-labeled neurons. *, Lateral ventricle.

Recovery of Function. To determine whether there is a functional correlate of the repopulation of neural cells in the striatum observed in the morphological and immunocytochemical studies, we carried out two sets of behavioral tests in some of these animals (Table 1). One group received a unilateral 6-OHDA lesion of the SN-VTA, and 14 days later, they received a 14-day continuous infusion of either aCSF or TGFα into the ipsilateral striatum. Rotational behavior in response to systemically administered apomorphine was tested before the lesion and then subsequently weekly for 4 weeks, both before and after the TGFα/aCSF infusions. In this rodent model of Parkinson's disease, there was a statistically significant ($P < 0.05$, two-tailed *t* test) 31.5% improvement in the rotational behavior of the TGFα versus aCSF-treated control animals.

A separate group of animals (individual animal data not shown) received TGFα, aCSF, or no infusions starting contemporaneously with the 6-OHDA lesion, as opposed to 2 weeks after the 6-OHDA lesion. In the groups that received either no infusion or an aCSF infusion, contralateral rotational behavior in response to an apomorphine injection was observed at each of 3- to 4-week intervals after the day when the animals received both the 6-OHDA injection and either aCSF infusion or no striatal infusion at all. In contrast, 10 of the 11 TGFα-treated animals did not exhibit rotational behavioral asymmetry following apomorphine injections during the entire 4-week period. Other motor behaviors such as exploratory behavior in the cage and consummatory behavior, on the other hand, did not appear to be adversely affected in the TGFα-treated animals.

Table 1. Behavioral results in Parkinson's disease model

	Rotations/5 min		
Animal	Preinfusion	Postinfusion	% change
TGFα			
1	66.5	14.5	−78.2
3	48.0	14.5	−69.8
4	60.0	25.0	−58.3
5	60.0	40.0	−33.3
6	58.5	39.5	−32.5
7	163.5	140.5	−14.1
8	50.0	49.0	−2.0
9	33.0	33.0	0.0
11	103.5	185.7	79.4
Mean	71.4	60.2	−15.8
SD	37.2	57.1	
aCSF			
1	66.0	45.0	−31.8
2	158.5	151.0	−4.7
3	42.0	41.0	−2.4
4	40.0	40.0	0.0
5	71.0	71.0	0.0
6	94.0	96.0	2.1
7	38.0	41.0	7.9
8	144.5	158.5	9.7
9	63.5	100.5	58.3
10	101.0	162.0	60.4
11	84.0	138.0	64.3
Mean	82.0	94.9	15.7
SD	40.4	50.5	
t test	(2-tailed)		0.046

Apomorphine-induced rotations in 6-OHDA-lesioned animals, and 14 days later infused for 14 days with aCSF (control) or TGFα (experimental group). The order of animals is by descending order of % change of rotation. The percent improvement of rotational behavior (three 5-min periods) between the TGFα-treated animals and aCSF-treated animals was 31.5% (significant at $P < 0.05$, two-tailed *t* test). The TGFα animals received either 50 or 100 μg TGFα. Animals receiving 10 μg TGFα did not show significant improvement and were not included in this analysis.

Discussion

These findings provide evidence of induced coordinated proliferation, directed migration en masse, and phenotypic differentiation into TH-positive neurons and DAT (therefore, likely dopaminergic) of neural stem cells and their progenitors in the mammalian central nervous system *in vivo*. The parallel morphological and behavioral rotation experiments, coupled with the finding of newly generated (BrdUrd positive) and phenotypically specialized (TH-, DAT-positive dopamine neurons), indicate the usefulness of exogenous TGFα administration in the amelioration and reversal of symptoms of Parkinson's disease and other neurodegenerative disorders, as well as acute central

nervous system injury, for example, due to trauma and stroke. We found that the combination of TGFα infusion and endogenous stimuli arising from the injury signals in experimental animals resulted in a massive proliferation of the cells of the SVZ, followed by a directed migration en masse toward TGFα infusion sites in the striatum, septum, and external capsule or cortex. Further, we demonstrated a progressive pattern of proliferation, migration, maturation, and differentiation of newly generated cells, leading to neuronal and glial phenotypes with "spontaneous" differentiation and phenotypic specialization of some neurons, correlated with a desirable functional result (i.e., reversal of motor dysfunction).

The embryonic germinal layer of the central nervous system that is retained in the adult SVZ throughout the neuraxis is responsive to microenvironmental signals and can proliferate and differentiate in response to TGFα and other growth factors (19). Developmental changes in expression of the type I family of tyrosine kinase receptor family in neuroprogenitor cells have been shown to influence their proliferation, migration, and differentiation (16, 20). Both EGF and TGFα, members of the EGF family that bind to the other members of this family (Erb1 and possibly others) (21, 22), are present in the basal ganglia (23–26), although TGFα mRNA expression has been shown to greatly exceed that of EGF (27). TGFα has been shown to influence proliferation and migration of ganglionic eminence cells in the embryo (28) and proliferation of SVZ cells *in vivo* (29) and *in vitro* (30), a mechanism mediated via the EGF receptor (12, 31). The EGFr family of receptors is expressed in striatal and SVZ cells in both adult and developing brains (26, 32, 33). The observed increased expression of EGFr mRNA in the early stages of TGFα infusion in this study is consistent with a TGFα-dependent, EGFr-mediated mechanism of progenitor cell proliferation, as has been shown in other studies (31, 34).

Although progenitor cell proliferation in response to growth factors has been demonstrated *in vitro*, it has become clear that *in vivo* differentiation and migration requires additional microenvironmental signals. *In vitro* cell proliferation and migration and differentiation has been shown in response to microenvironmental cues provided by the substrate (e.g., fibronectin and/or integrins) (35) or altered in relation to neighboring cells, implying a cell–cell paracrine or contact mechanism (36). *In vivo*, however, it appears that microenvironmental manipulation using injury paradigms may also be effective in stimulating proliferation and migration of neural precursors, as demonstrated using several injury models such as apoptosis (17), ischemia (37, 38), and chemical toxicity (39, 40). The injury signal produced by the 6-OHDA lesion and infusion cannulae in our study may similarly result in a cascade of timed microenvironmental stimuli that may promote the migration en masse of neural progenitors from the SVZ in response to TGFα. Although other factors may ultimately interact to produce optimal PMD under various experimental and clinical conditions, it is surprising that application of a single neurotrophic factor (TGFα) in an injured brain region is sufficient to effect such significant repair mechanism(s) with positive functional results. TGFα may have several mechanisms of action, perhaps through interactions with multiple TGFα/EGF receptor subtypes (41), which lead to behavioral recovery, such as PMD of stem and progenitor cells, which replace lost circuits and functions; induction of new phenotypic expression in preexisting cells; and neuroprotection against cytotoxic or apoptotic signals. TGFα may be an important endogenous trophic factor in both central and peripheral tissues throughout development, adulthood, and in response to injury and degeneration of tissue. For example, an increase in TGFα levels has been measured in the striata of some Parkinson's disease patients (42, 43). Perhaps the TGFα released endogenously in degenerative disorders is not at a high enough concentration to offset the progressive neural loss with a compensatory stimulation of PMD. Exogenously administered TGFα over a threshold level in patients with Parkinson's disease or other chronic and acute neural damage may then lead to system-specific regeneration and protection of neural circuitry, as well as reversal of clinical symptoms.

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